



Rhabdomyosarcoma: the Search for New Genes and Pathogenetic Mechanisms

Pediatric Research Grand Rounds, 5/18/11

*Children's at Egleston, Children's Healthcare of Atlanta,
& Emory University*

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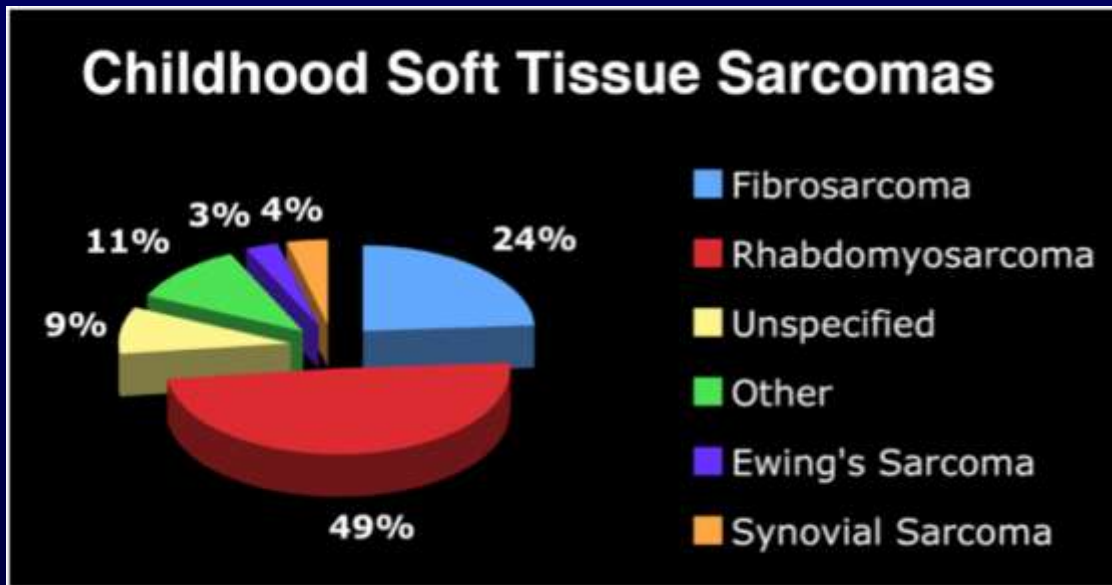
Departments of Pathology and Molecular Biology

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Childhood Development and Disease

- “Children are not just little adults”- includes childhood cancer
- Children do not suffer from the typical tumors of adulthood (*e.g.*, epithelial carcinomas)
- Instead, pediatric solid tumors are typically of connective tissue (sarcomas) or neuroectodermal origin
- Why do sarcomas prefer childhood tissue?

Genetics of Childhood Sarcomas



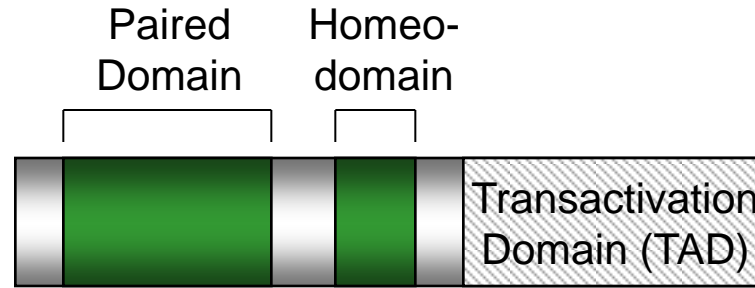
Adapted from SEERS program data, NCI

- Childhood sarcomas are genetically distinct diseases
 - Rhabdomyosarcoma (RMS)
 - $t(2;13)$, $t(1;13)$; PAX-FKHR
 - Ewing's Sarcoma
 - $t(11;12)$; EWS-FLI1
 - Synovial Sarcoma
 - $t(X;18)$; SYT-SSX
- Lack of true/efficient animal models
 - Toxicity of fusion oncoproteins
 - Unknown cell/tissue of origin
- RMS is a tumor of skeletal muscle-type histology, and most common
- PAX-FKHR RMS is notoriously aggressive

Rhabdomyosarcoma and PAX-FKHR

The PAX-FKHR Protein Fusion

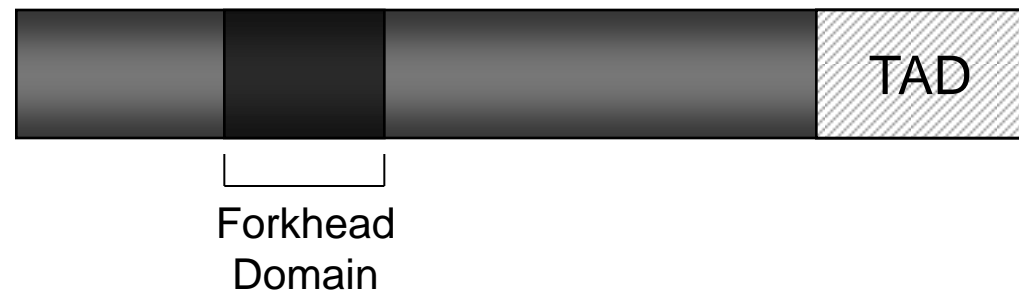
PAX3/7
(Chromosome 2/1)



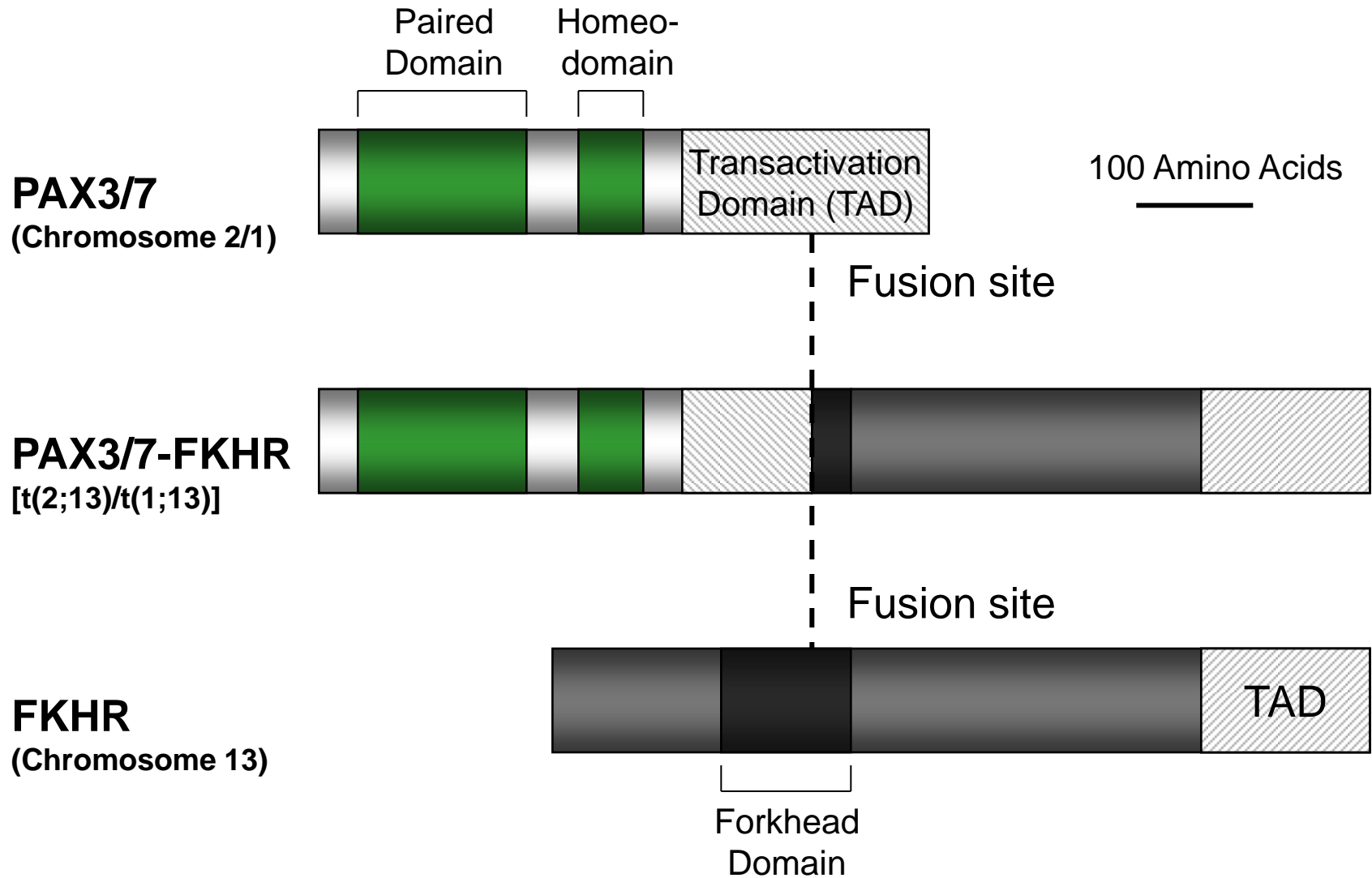
100 Amino Acids



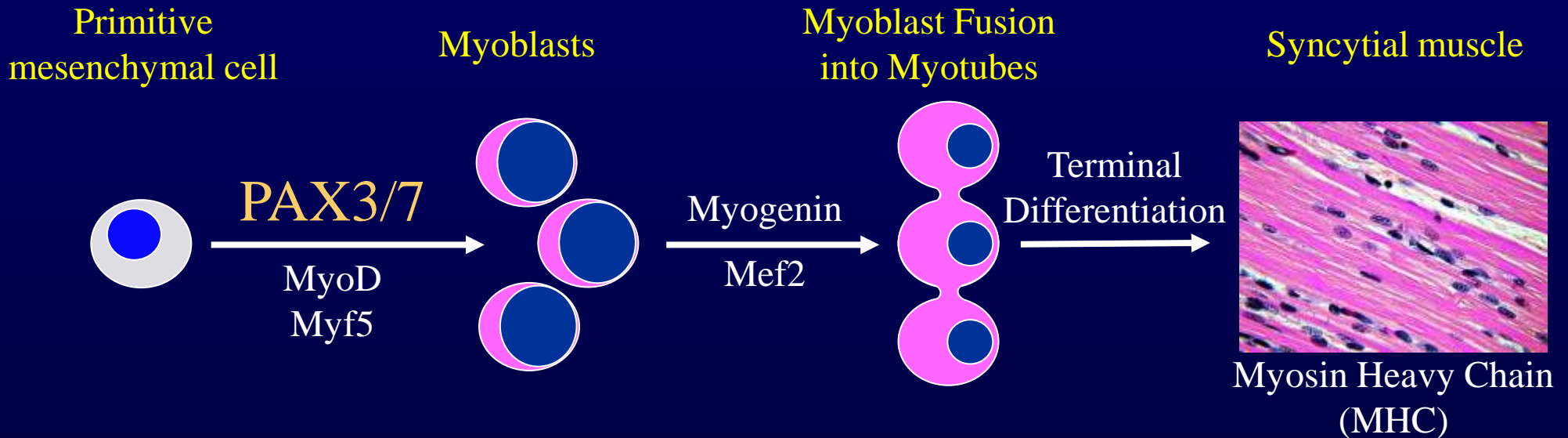
FKHR
(Chromosome 13)



The PAX-FKHR Protein Fusion

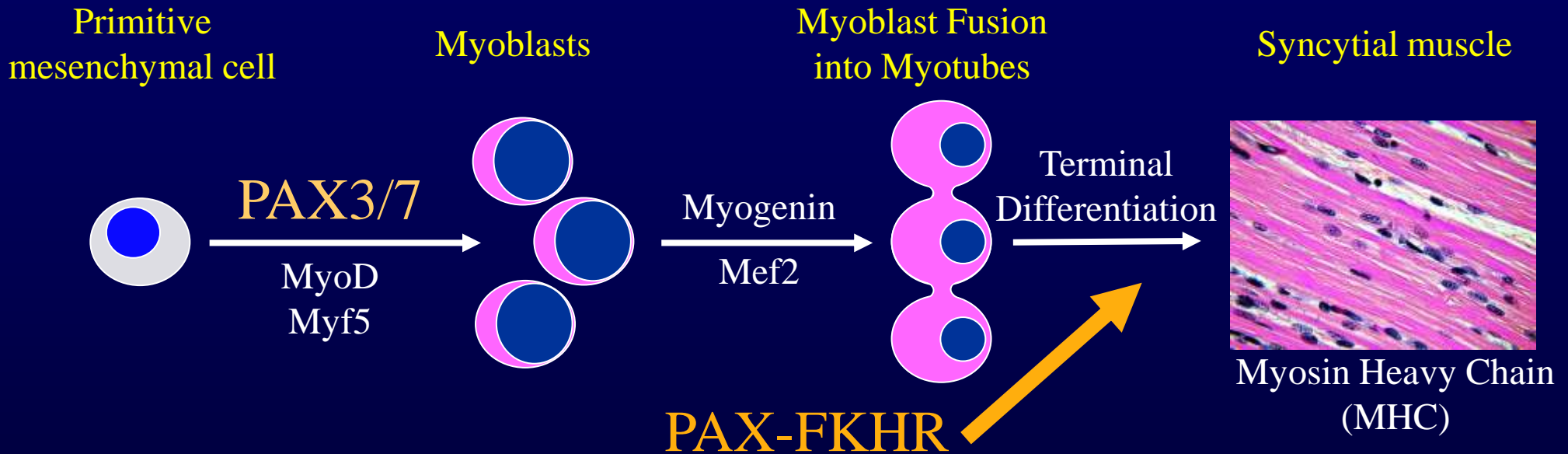


Myogenesis: Myoblasts, Cell-Cell Fusion, and then Muscle



- Little is known regarding PAX3/7 gene targets and cofactors
- Gene redundancy in mammals
- The underpinnings of PAX-FKHR pathogenesis remain poorly understood

Myogenesis: Myoblasts, Cell-Cell Fusion, and then Muscle



- Keller et al., *Genes Dev*, 2004- PAX3-FKHR expressed in differentiating muscle caused RMS tumorigenesis
- However, suboptimal tumor incidence and latency for *in vivo* profiling of PAX-FKHR cell biology and pathogenesis

A Drosophila PAX-FKHR Model System



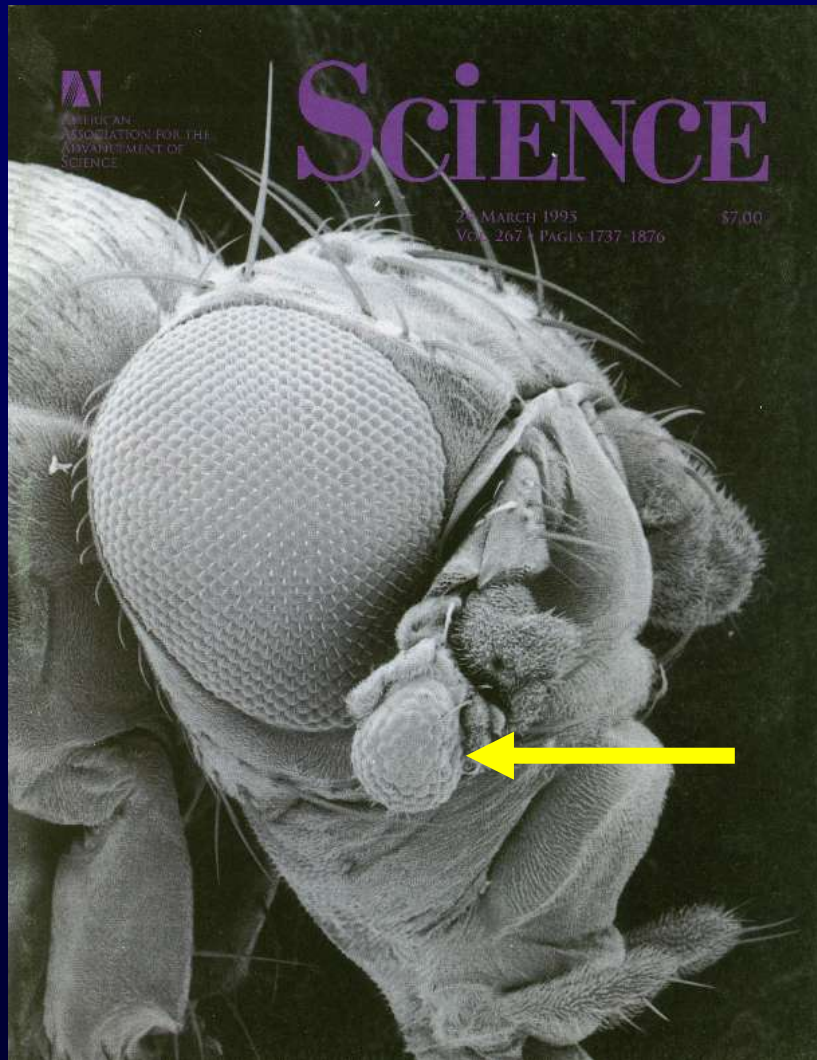
A *Drosophila* PAX-FKHR Model System



Drosophila melanogaster
(<http://flymove.uni-muenster.de>)

- Entire, intact organisms can be examined by conventional microscopy
- Fluorescent protein reporters
- Conditional expression of transgenes
- Unbiased forward genetic screens
- **Rich conservation of mammalian genes- particularly PAX**

Human and Fly PAX



- PAX6- master regulator of eye development in flies and mammals
- PAX6 misexpression in flies causes ectopic eyes
- True for BOTH fly and mammalian PAX6
- Why not PAX3/7-FKHR?

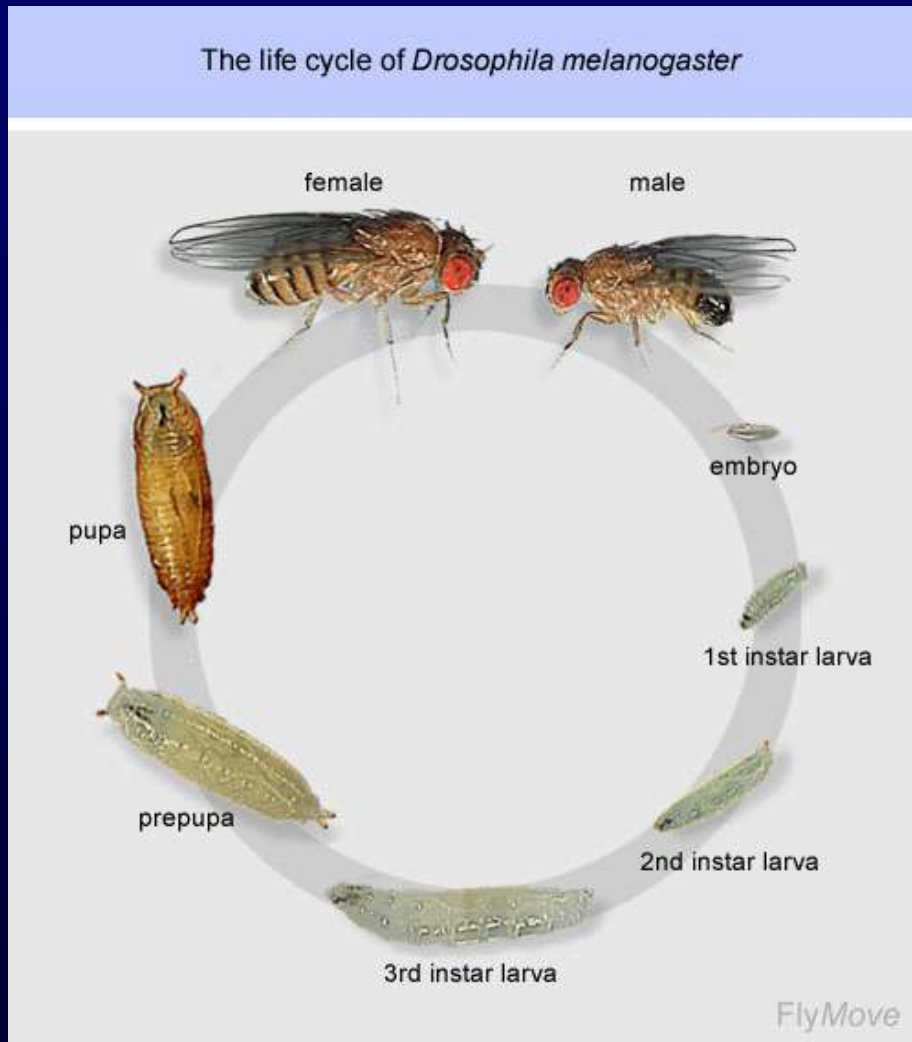
Halder *et al.*, 1995

Human and Fly Pax3/7

Gsb-Dm	1	---M A V S A L N M T F Y F E G -Y P F Q -----G Q G R V N Q L G G V F I N G R P L P N H I R H K I V
Gsb Neuro-Dm	1	---M D M S S A N S L R P L F F A G -Y P F Q -----G Q G R V N Q L G G V F I N G R P L P N H I R L K I V
PAX3-Hs	1	M T T L A G A V P R M M R P E G C O N Y P R S G F P L E V S T P L G Q G R V N Q L G G V F I N G R P L P N H I R H K I V
PAX7-Hs	1	M A A L P G I V P R M M R P E G C O N Y P R T G F P L E V S T P L G Q G R V N Q L G G V F I N G R P L P N H I R H K I V
Gsb-Dm	46	E M A A A G S R P C V I S R Q L R V S H G C V S K I L N R F Q E T G S I R P G V I G G S K P N-V A T P D N E S H I E E
Gsb Neuro-Dm	47	E M A A A G S R P C V I S R Q L R V S H G C V S K I L N R Y Q E T G S I R P G V I G G S K P N-V T P E P E T H I E
PAX3-Hs	61	E M A H H G S R P C V I S R Q L R V S H G C V S K I L C R Y Q E T G S I R P G A I G G S K P NQ V I T P D N E K I E E
PAX7-Hs	61	E M A H H G S R P C V I S R Q L R V S H G C V S K I L C R Y Q E T G S I R P G A I G G S K P NQ V A T P D N E K I E E
Gsb-Dm	105	L K Q S L P G I F S W E I R A K L E A G V C D R K N A P S---V S S I S R L L R G S S G S C T S-----
Gsb Neuro-Dm	106	L R K E N E S I F S W E I R K L K K G F A D P---P S ---T S S I S R L L R G S D R G S E D G -----
PAX3-Hs	121	Y K R E N P G I F S W E I R D K L K D V C D R I T V P S ---V S S I S R L L R S K F G K G E E E E A D L E R K E A F
PAX7-Hs	121	Y K R E N P G I F S W E I R D L L K D G H C D R S T V P S G L V S S I S R L L R I K F G K K E E E---D E A D K K E D
Gsb-Dm	153	-----H S I D G I L G G G A G S V G S E D E S E D D A E P S V Q L K R K Q R R S R T T F S N D Q I D E L E R I F
Gsb Neuro-Dm	151	-----R K D Y I I G I L G G R D S -----D I S D T E S E P G I P L K R K Q R R S R T T F T A E Q L E L E R A F
PAX3-Hs	179	E S E K K A K H S I D G I L S E R A S A P Q S D E G S D I D S E P D L P L K R K Q R R S R T T F T A E Q L E L E R A F
PAX7-Hs	179	D G E K K A K H S I D G I L G D K G N R ---L D E G S D V E S E P D L P L K R K Q R R S R T T F T A E Q L E L E K A F
Gsb-Dm	205	A R T C Y P D V Y T R E E L A Q S T L T E A R V Q V W F S N R R A R R R K Q L N -----T Q Q V E S F A
Gsb Neuro-Dm	202	A R T C Y P D V Y T R E E L A Q S T L T E A R V Q V W F S N R R A R R R K H S G -----S N S C L S P M N
PAX3-Hs	239	E R T H Y P D I Y T R E E L A Q R A K L T E A R V Q V W F S N R R A R R K Q A G A N Q L A F N H L L P G G F P P T A
PAX7-Hs	237	E R T H Y P D I Y T R E E L A Q R T K L T E A R V Q V W F S N R R A R R K Q A G A N Q L A A F N H L L P G G F P P T A
Gsb-Dm	254	P T S T S F G A T E T T S A A P A P N M G-----M S I Y S S Q S W E S S C A Y E N H A A Y G S V A S M S P A S S
Gsb Neuro-Dm	253	S G S S N V G V G V L S G A T P L G Y C P L G---V G S M A G Y S P A P G T T A T G A G N D G V H H A A H A P S S H
PAX3-Hs	299	M P T L P T Y Q L S E T S Y Q P T S I P Q A V S D P S S T V H R P Q L P P S T V H Q S T I P S N P D S S A Y C L F S
PAX7-Hs	297	M P T L P P Y Q L P D S T Y P T T I S Q D G G---S T V H R P Q L P P S T M H Q G L A A A A A A A D T S S A Y G
Gsb-Dm	308	T S G T S S A A H S P V Q T Q A Q-----
Gsb Neuro-Dm	312	H S A A T A A A A A H H H T Q L G G Y D L V C S A A ---
PAX3-Hs	359	T R H G F S S Y T D S F V P P S G P S N P M N P T I G N
PAX7-Hs	354	A R H S F S S Y S D S F M N E A A P S N H M N P V S -N

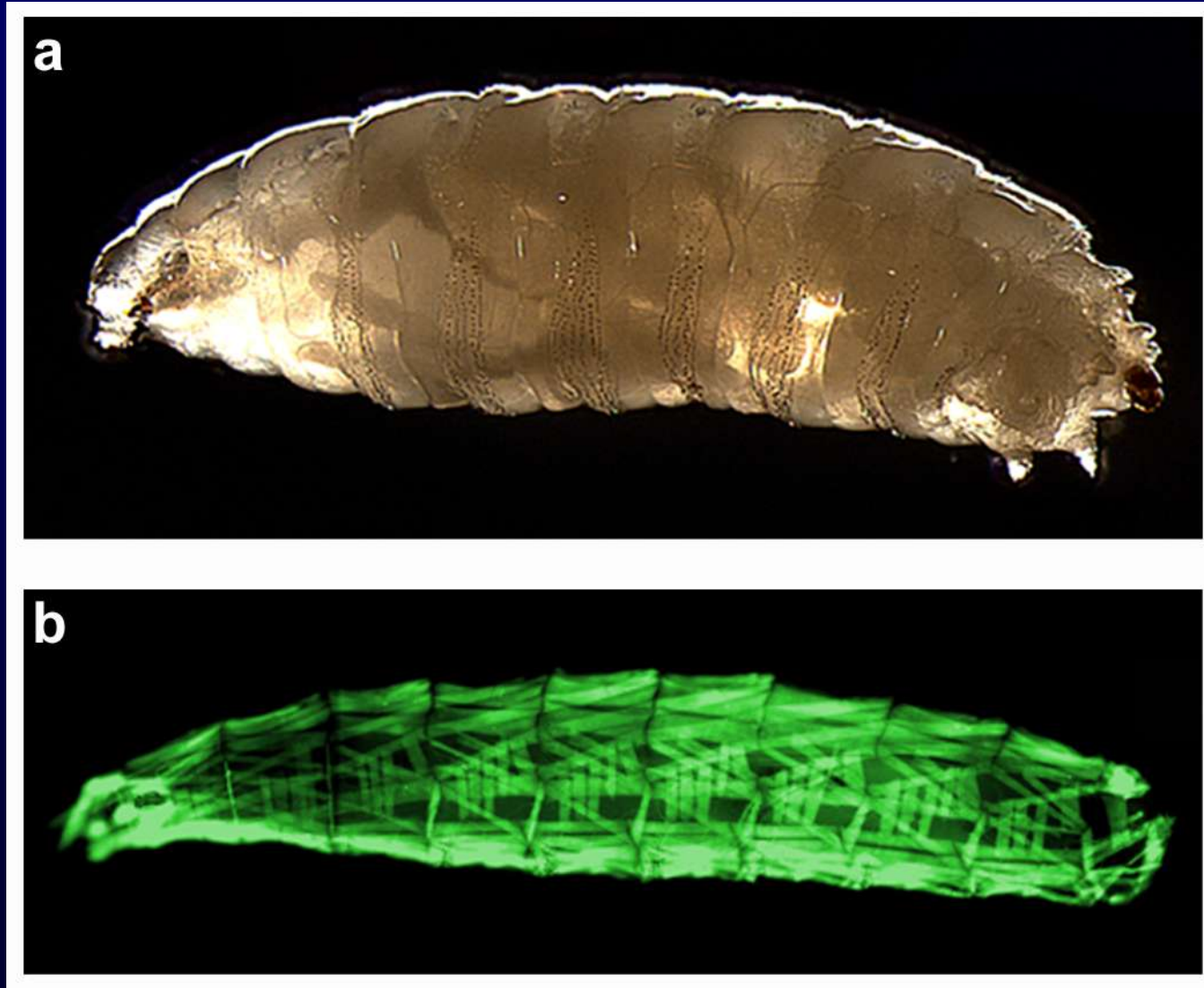
- *Drosophila* and mammalian PAX3/7 are structurally and functionally conserved
- PAX3- e.g., Xue and Noll, *Development*, 1996
- Both mammalian and fly PAX3/7 participate in muscle biology
- Again, why not PAX-FKHR?

PAX-FKHR in *Drosophila* Muscle

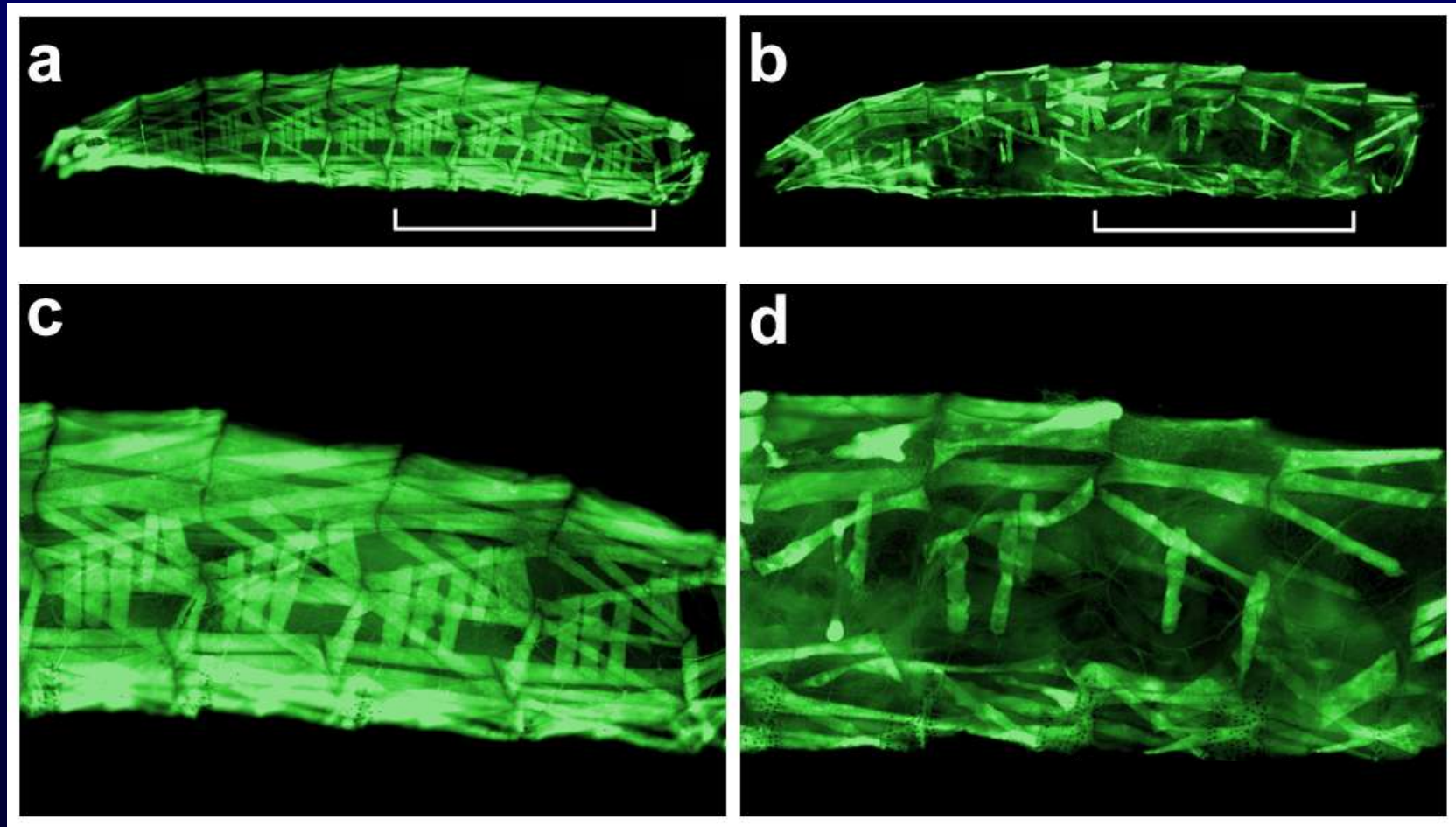


- *Myosin heavy chain (MHC)* regulatory elements to direct *PAX-FKHR* expression in differentiated syncytial muscle
- Expression is predominantly post-embryonic
- Targets larval juvenile muscle undergoing physiologic growth

Real-Time Examination of *Drosophila* Muscle



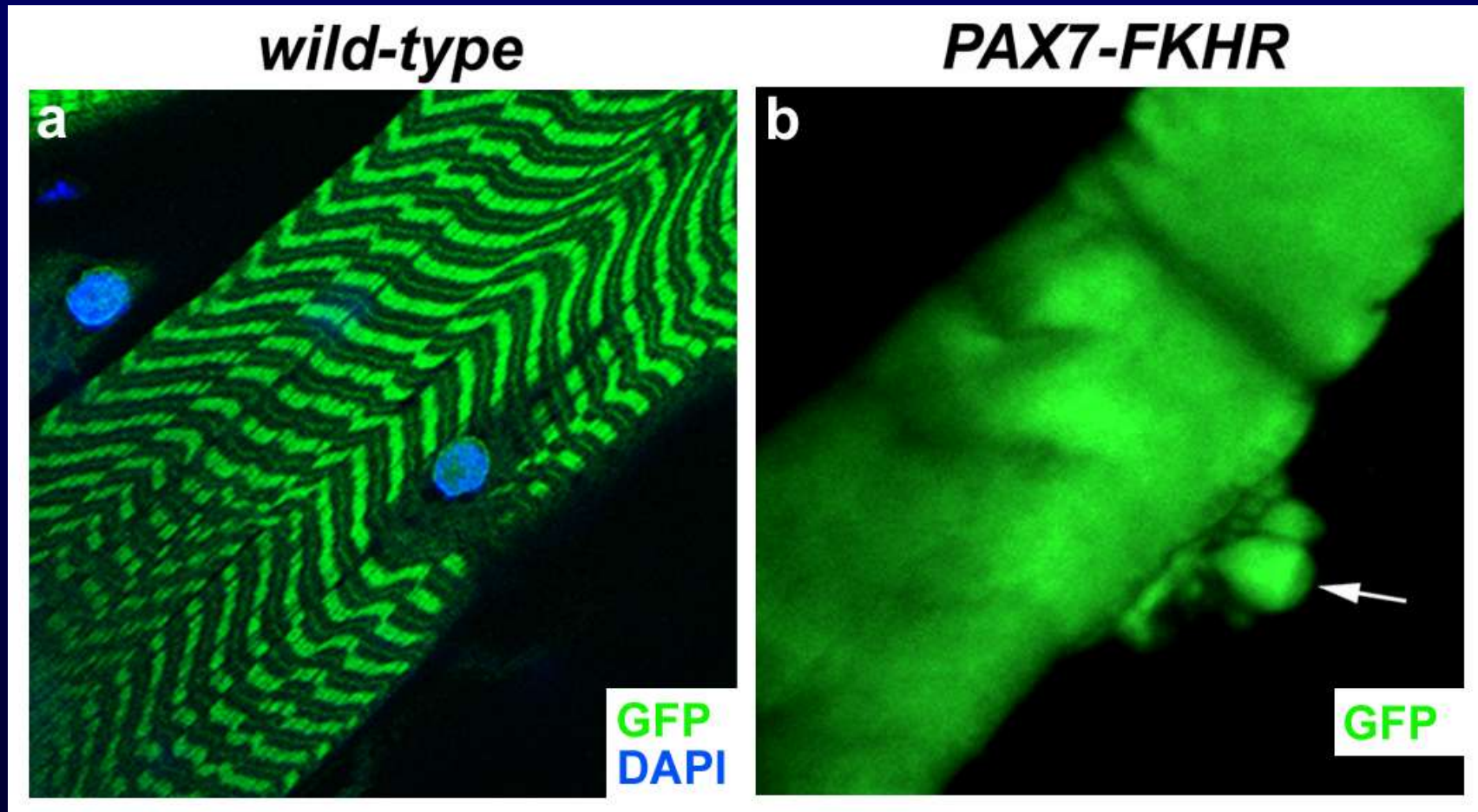
PAX-FKHR Compromises Muscle Patterning



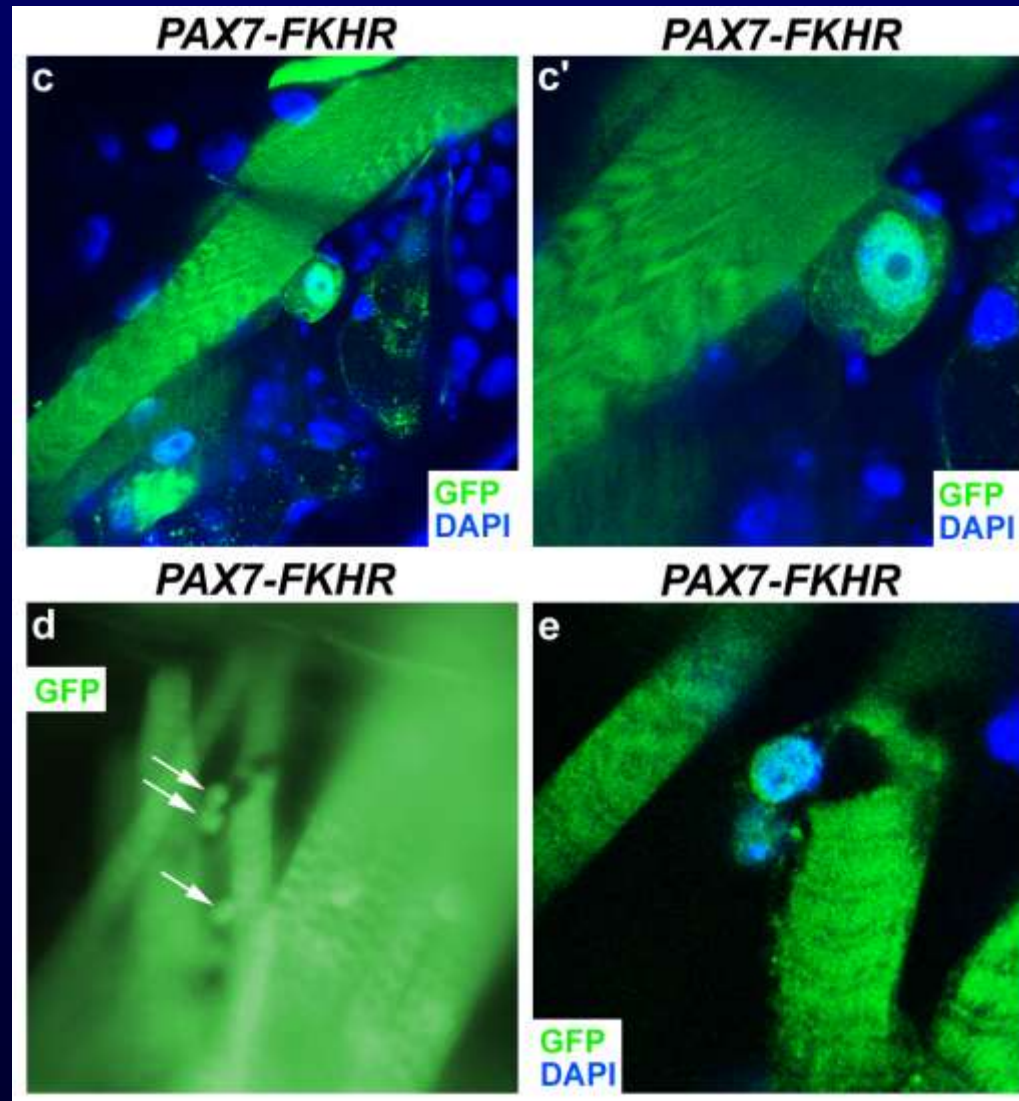
MHC>>GFP

*MHC>>GFP,
PAX-FKHR*

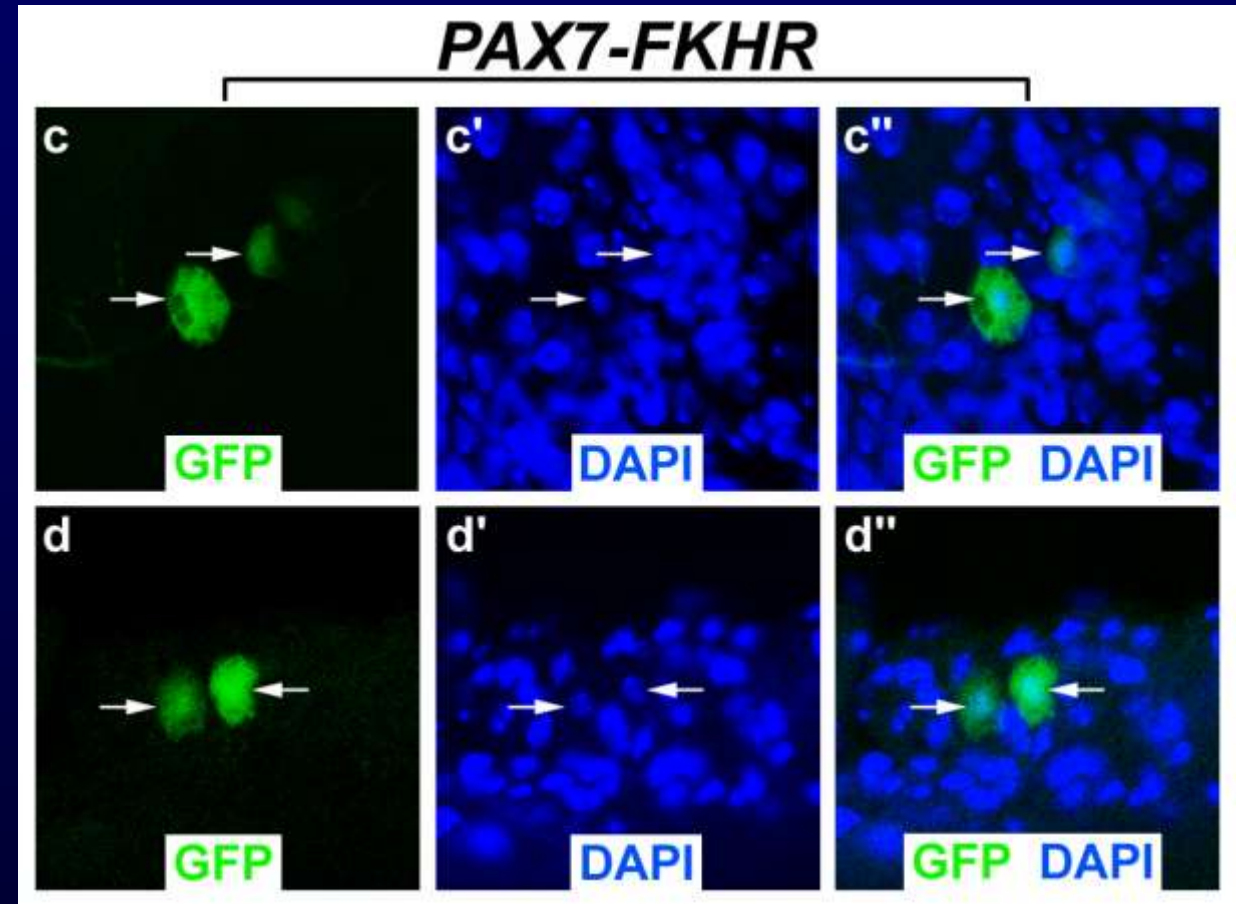
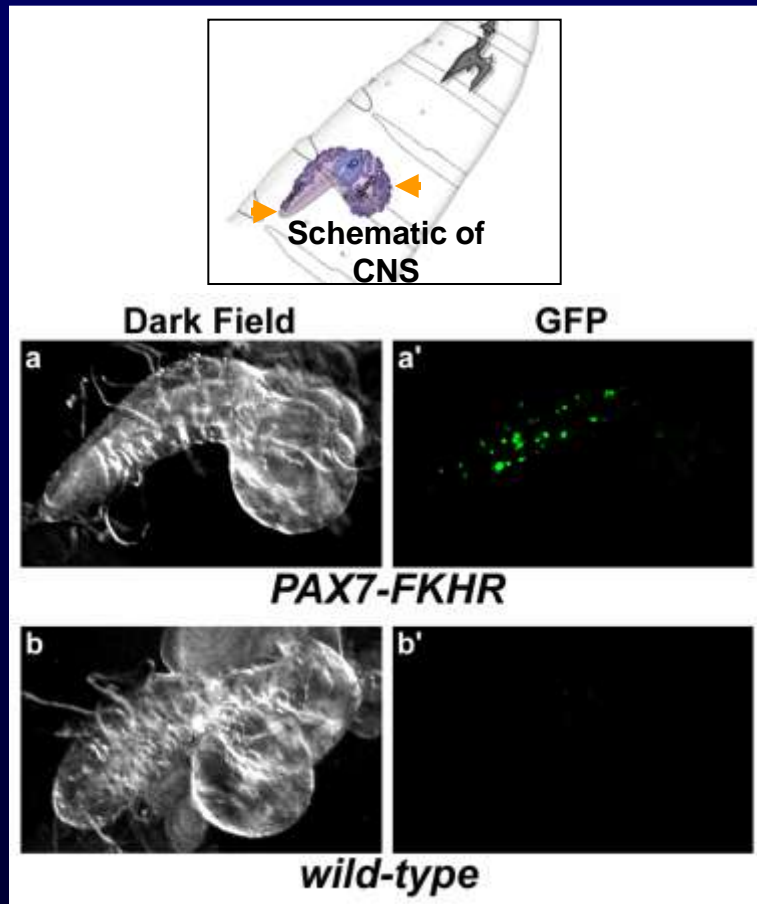
PAX-FKHR Muscle Demonstrates Fusion Defects



PAX-FKHR Muscle Demonstrates Fusion Defects



PAX-FKHR Myogenic Tissue in the Larval Central Nervous System



“Ok, but it is screenable?”
Eric Olson, circa 2007

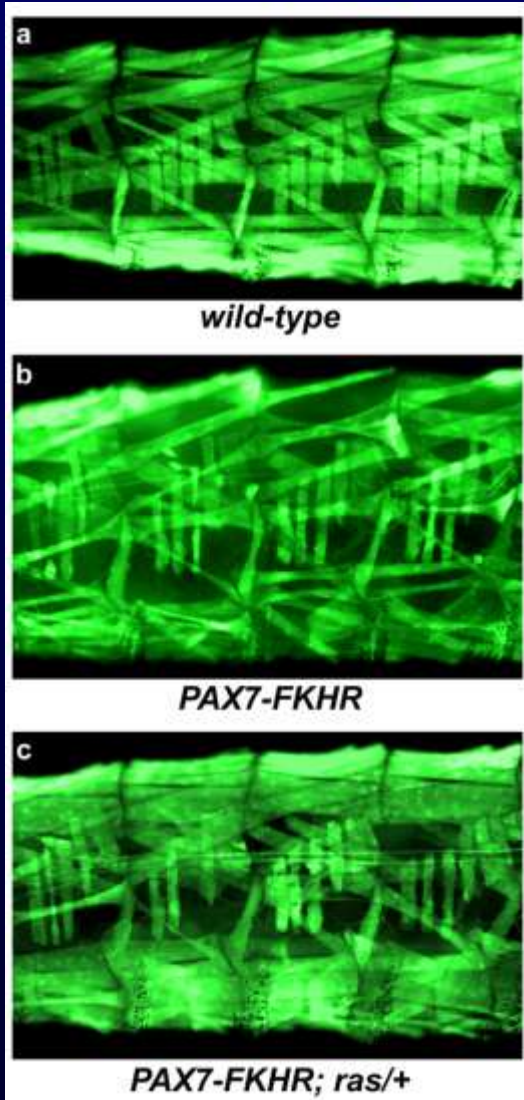
An Invertebrate Genetic Approach to the Study of PAX-FKHR



Drosophila melanogaster
(<http://flymove.uni-muenster.de>)

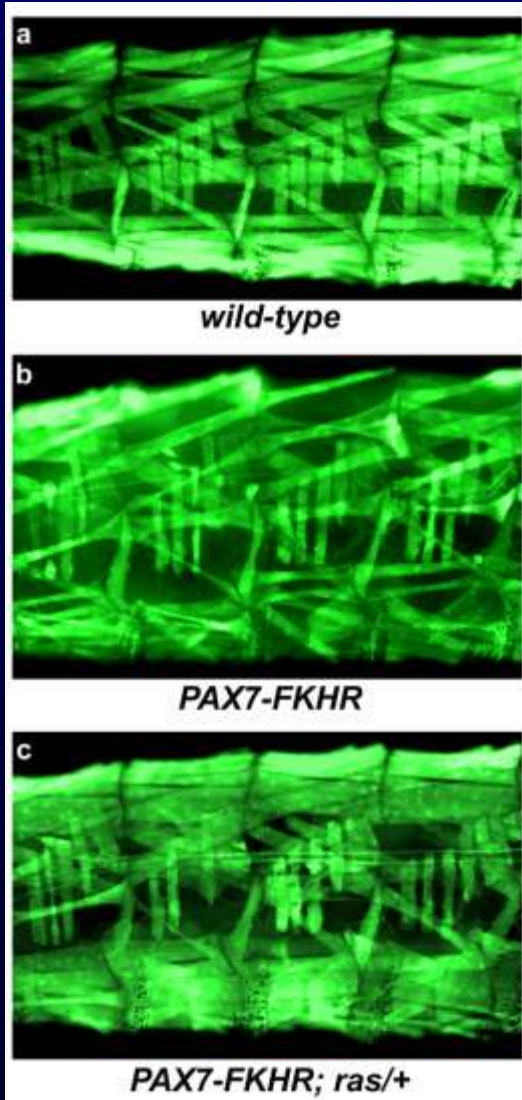
- Entire, intact organisms can be examined by conventional microscopy
- Fluorescent protein reporters
- Conditional expression of transgenes
- **Unbiased forward genetic screens**
- Rich conservation of mammalian genes- particularly PAX

Ras Loss-of-Function Suppresses *PAX-FKHR* Activity and Rescues Lethality



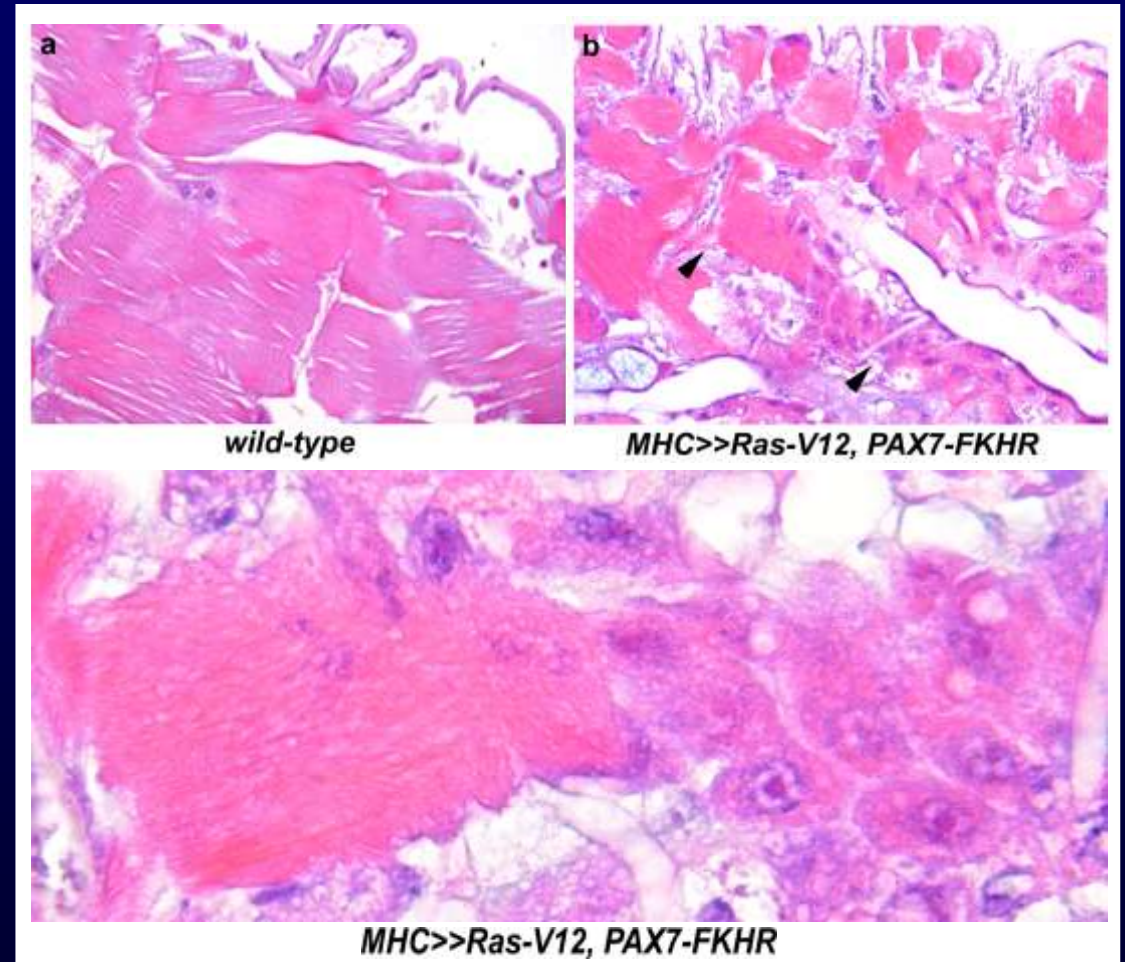
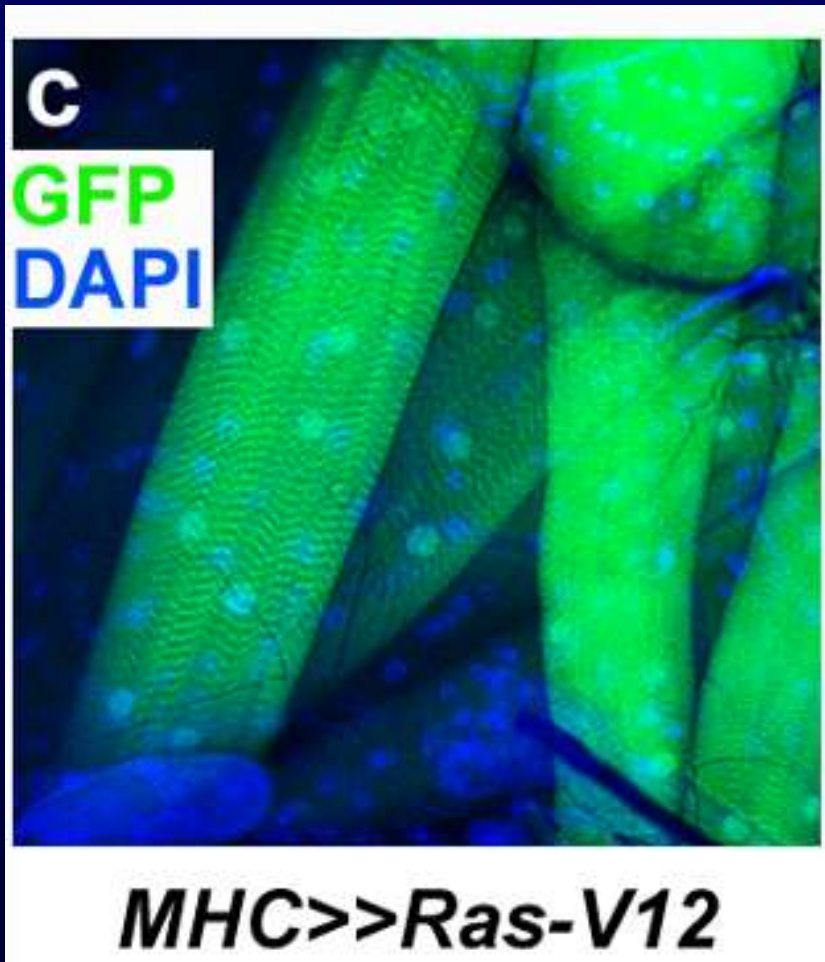
- ✓ Tested loss-of-function *ras* mutation for *PAX-FKHR* suppression
- ✓ Dominantly suppressed *PAX-FKHR* pathogenicity in muscle
- ✓ Rescues *PAX-FKHR* larval lethality to adult viability

Ras Loss-of-Function Suppresses *PAX-FKHR* Activity and Rescues Lethality



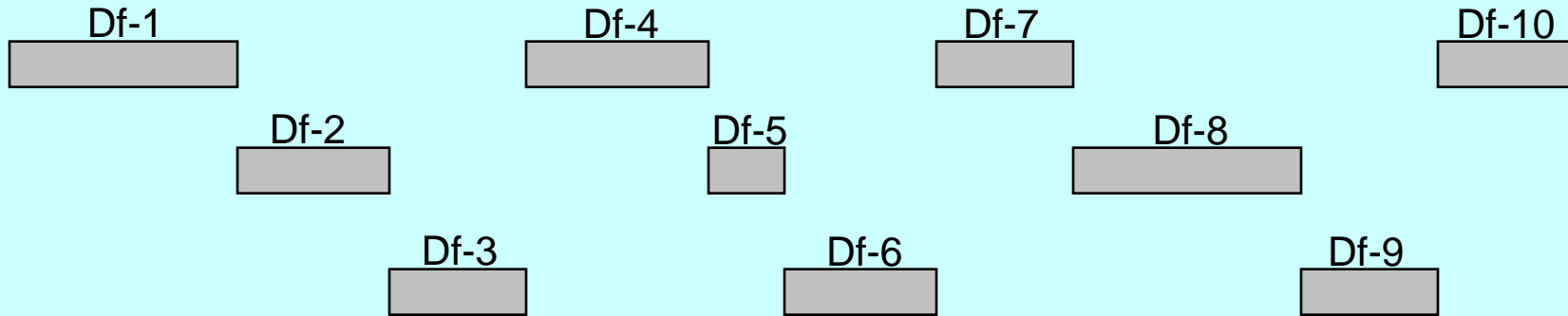
- ✓ Tested loss-of-function *ras* mutation for *PAX-FKHR* suppression
- ✓ Dominantly suppressed *PAX-FKHR* pathogenicity in muscle
- ✓ Rescues *PAX-FKHR* larval lethality to adult viability

Ras^{V12} Gain-of-Function and *PAX-FKHR* muscle



Deficiency Screening

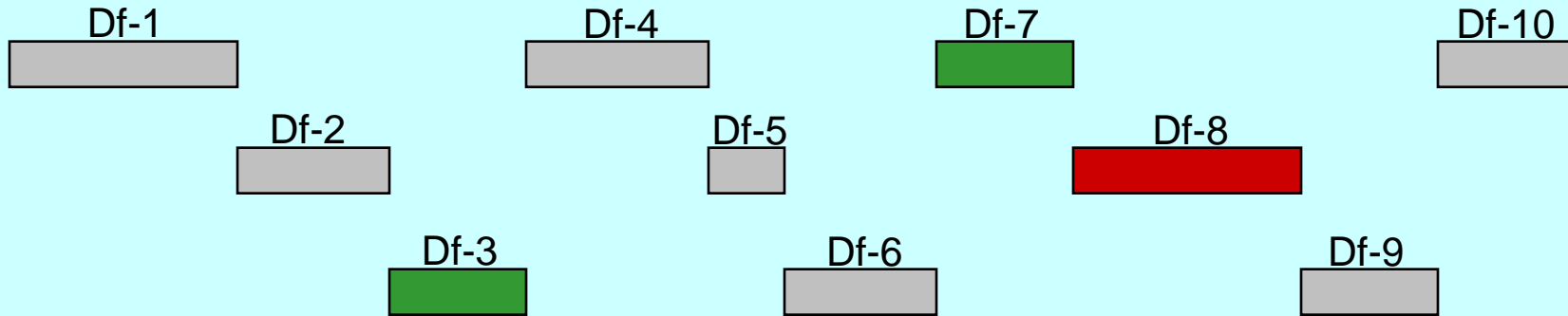
Chromosome 2



Df = Deficiency

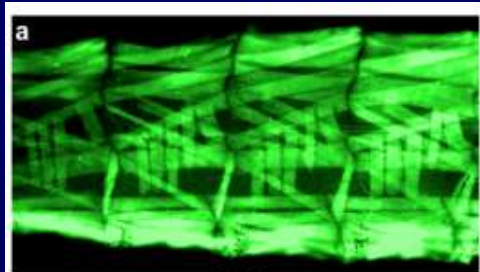
Deficiency Screening

Chromosome 2

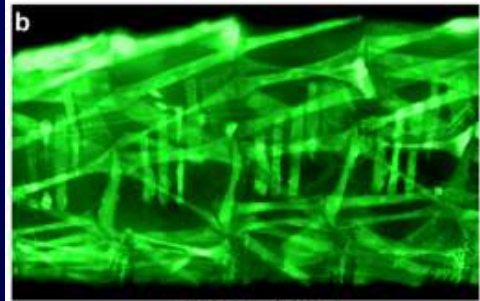


Df = Deficiency

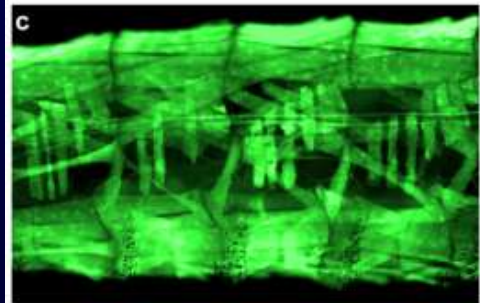
PAX-FKHR Deficiency Screening



wild-type



PAX7-FKHR



PAX7-FKHR; ras/+

Deficiency Line	# Flies Examined	"Expression-OFF"	"Expression-ON"
		<i>Df</i> <i>MHC>>PAX7-FKHR</i>	<i>Df</i> <i>MHC>>PAX7-FKHR</i>
"Expected" (Mendelian Ratios)	-	50	50
None (control 1)	75	71	29
None (control 2)	58	72	28
<i>Df(2L)pr-A16</i>	32	66	34
<i>Df(2L)C'</i>	42	69	31
<i>Df(2L)BSC16</i>	53	64	36
<i>Df(2L)E110</i>	49	47	53
<i>Df(2L)BSC36</i>	58	48	52
<i>Df(2L)ast2</i>	49	88	12
<i>Df(2L)BSC30</i>	37	86	14

Non-modifier
 Suppressor
 Enhancer

PAX-FKHR Screening and Results

957;9A17-1B	2363	0	194	194	0%
957;9D11;9P15	4832	0	225	225	0%
916;8A02	2577	0	115	115	0%
75A6-7;75C1-2	2408	0	217	217	0%
646;9C	3096	0	51	51	0%
75H10-11;75A1-5	6734	0	77	77	0%
5689;13;7D1-12	1463	1	74	75	1%
85A2;85C1-2	1962	3	189	192	2%
79C1-3;70D4-5	3124	1	150	151	2%
49C1-4;49C23-D2	442	2	88	90	2%
65A2;65B1	2021	2	85	87	2%
60E2-3;60E11-12	2471	5	140	145	3%
7174-4;72D1-10	1105	5	124	129	4%
6376-7;64C13-15	3886	4	97	101	4%
84A3-4;84E1-4	2588	4	71	75	4%
49A4-11;49E7-F1	724	4	128	134	4%
93B6-7;93D2	1340	4	103	107	4%
5702-6;58D1	5046	8	140	148	4%
88F7-11;89A1	183	13	175	188	7%
84B2;84B17	3096	5	64	71	7%
8173-6;8275-7	1518	9	112	117	8%
60C8;60B8	9005	10	112	124	8%
53D9-E1;54B5-10	7443	12	112	124	10%
54C1-4;54C14	6516	10	100	109	10%
85D8-12;85E7-F1	1871	18	174	190	10%
97B;98A3	822	18	152	170	10%
41A;41A	739	22	167	189	12%
607;6075	4963	11	89	91	12%
66B8-9;66C9-10	1541	19	129	148	13%
63B8-6;64B8-9	463	42	276	318	13%
84A5;84D9	1842	18	106	124	15%
8278-10;83A1-3	5694	34	196	230	15%
62A10-81;62D2-5	600	14	80	94	15%
96A1;96FJ0	9500	17	93	110	15%
773C-77F;78A	3127	35	189	225	16%
99D5-10;60B3-8	1882	18	94	112	16%
44D1-4;44F12	201	38	196	234	16%
56F12-14;57A4	6609	14	71	85	16%
99D5-6;99F1	2547	51	204	255	17%
95D8;95E5	7676	22	109	131	17%
95D3;95I	2546	41	107	148	17%
77	447	17	81	98	17%
53E4;53F6	7876	29	136	165	18%
96C9;96E2	7981	32	150	182	18%
7770;77E-9	4429	83	387	470	18%
67E3-7;68A2-6	6471	21	96	117	18%
48A3-4;8C6-8	1145	22	97	119	18%
96A1;97B1	5601	28	123	151	19%
84A1-2;84B1-2	1884	26	114	140	19%
8387;C1;83E6-D1	7443	32	140	172	19%
51D3-8;5275-9	3518	27	118	145	19%
78D2;78A2	8101	93	390	483	19%
42A1-2;42E6-F1	1007	28	116	144	19%
83D3-2;84A5	2393	19	78	97	20%
71E1-4;72D1-10	m118 3640	98	390	488	20%
87E1-2;98A	9929	46	176	222	21%
62A2;62A7	6766	103	389	492	21%
54B17-C;4;54C1-4	5680	34	126	160	21%
77E2-4;78A2-4	m118 1878	44	163	207	21%
73A3;74F	2998	39	134	173	21%
96E2;96E6	7882	37	160	184	23%
88E3;99A6-8	430	63	210	273	23%
95A2-4;95A8-B1	9497	43	141	184	23%
94E9;94E13	7990	37	118	155	24%
95E12;95D8	9795	52	155	207	25%
54B1-3;54B7-10	m118 7444	47	218	292	25%
60C5-6;60D9-10	2604	39	109	148	26%
66A17-10;66C1-5	5877	23	62	85	27%
68C8-11;69B4-5	2612	47	126	173	27%
88C1-8;81E1-5	1128	38	99	137	28%
77A1;77D1	2002	58	150	208	28%
77A1;77D1	8882	35	90	125	28%
7472;74A1	787	31	79	110	28%
69A4-5;69D4-6	5492	43	107	150	29%
85B1-2;85A	282	40	92	132	30%
83B4;83B6	8103	50	104	154	32%
85A;85F	1547	71	147	218	33%
95B1;95D1	7992	53	109	162	33%
69B6;70A1;70A1-2	6457	36	74	110	33%
88E3;99A6-8	430	38	78	116	33%
45E4;45E12	8410	43	86	129	33%
95A1-3;95D1-4	3909	43	85	128	34%
97A;98A2	11010	90	176	266	34%
657;66D10	1420	41	79	120	34%
54C-2;50B10	5886	48	88	136	35%
72C1-D1;73A3-4	2993	58	106	164	35%
88E3;99A6-8	430	39	70	109	36%
62B4-7;62D5-E5	2460	51	89	140	36%
46A;46A	1743	50	86	136	37%
84D1-2;84E5-7	6779	58	99	157	37%
45A6-7;45E2-3	4966	83	140	223	37%
56E11;56E16	7886	56	94	150	37%
42B3-5;42E15-18	1888	56	91	147	38%
78D4;78B	5126	42	67	109	39%
74D3-75A1;75B2-5	6411	53	84	137	39%
68A3-5;68A1-3	6511	38	60	89	39%
48C5-D1;48D5-E1	7149	87	109	169	40%
48E;49A	4966	56	85	144	40%
8274-8;82710-11	4787	59	87	146	40%
83799	41	59	100	161	41%
85C4-8;85D12-14	1756	61	85	146	42%
66D7-13;5499-12	6677	67	92	159	42%
66E1-2;67B3-3	7079	102	262	464	42%
6089;60C4	9691	76	103	179	42%
75A7-8;1;75B4-5	198	61	81	142	43%
88B1-2;88B3-5	7412	129	169	298	43%
44E10;45D1-E1	3201	46	60	106	43%
66D10-11;66E1-2	3024	51	63	114	45%
67A2;67D11-13	767	47	58	105	45%
95A4;95B1	7674	45	55	100	45%
59D;59D-11	2173	59	73	131	45%
50D1;50D7	6516	101	120	221	46%
95A5-4;95F4-6	6017	64	75	139	46%
50D4;50D4	7679	105	120	223	47%
95A5-7;95D6-11	2389	99	111	212	47%
48E12-14;48A11;86	5879	79	88	167	47%
54C8-D1;54E2-7	7441	98	109	207	47%
42E;44C	3368	59	64	123	48%
69D4-5;69F7-6	6356	62	67	129	48%
66E4-6;66F1-6	4900	43	45	88	49%
74C2-3;72B1-C1	6531	67	67	134	50%
78E;F1;80A2-3	668	32	31	63	51%
95A5-7;95C10-11	4940	58	52	110	53%
46C;47A1	1702	84	72	136	54%
50E-F1;51E2-4	6455	47	35	81	57%

- Screened ~75% of the *Drosophila* genome (156 *Df* stocks)

- 36 Suppressors, 30 enhancers

- Secondary screening for individual gene suppressors/enhancers

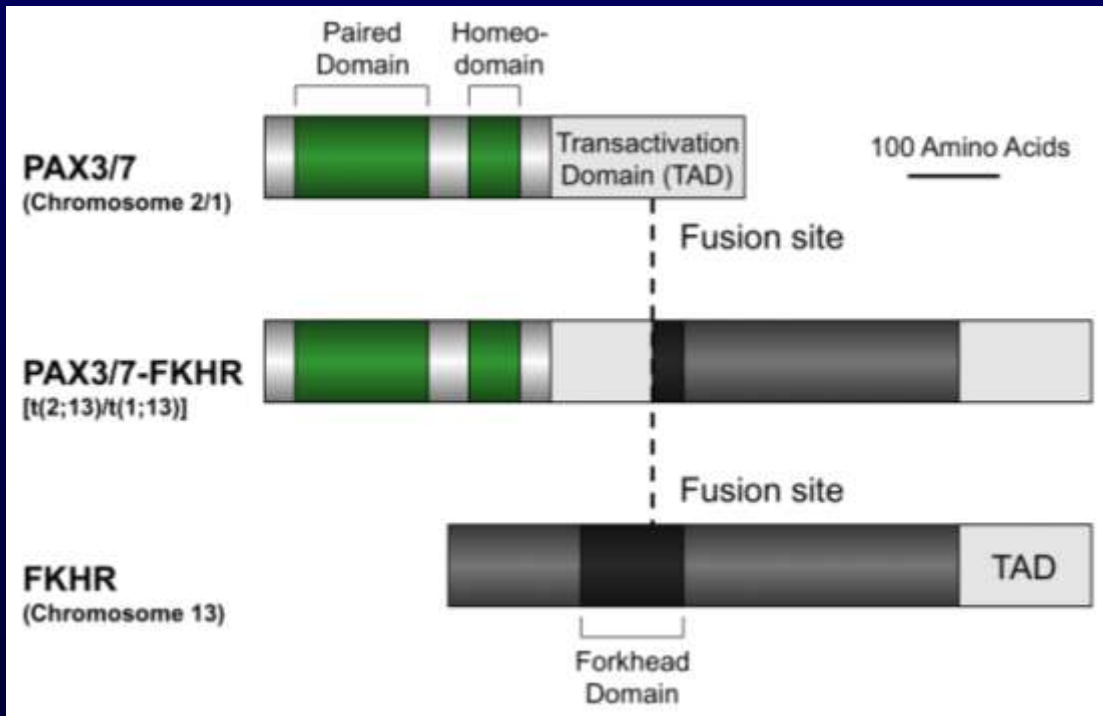
- Microarray analysis of wild-type versus PAX-FKHR muscle: ~750 genes differentially expressed

- Misexpressed genes = gene targets?

- Unaltered genes potential co-factors

Molecular Effectors of PAX-FKHR Pathogenicity

Molecular Effectors of PAX-FKHR Pathogenicity



1. Muscle Development effectors/regulators

✓ *Mef2*

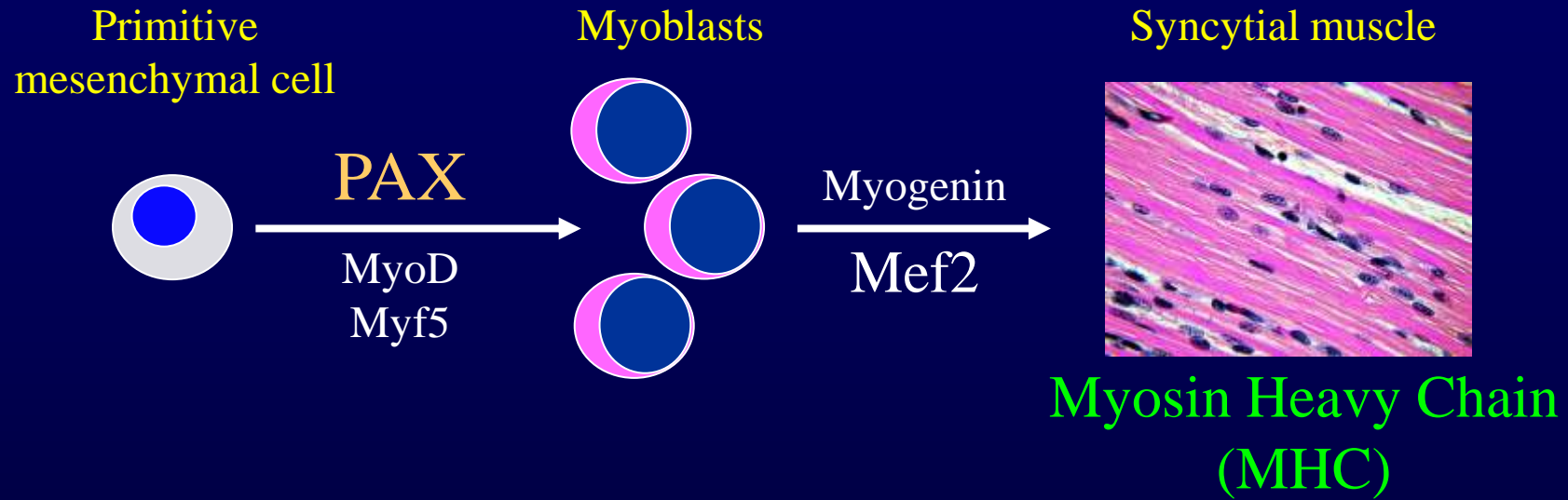
2. Myoblast fusion regulators

✓ *Antisocial*

3. Growth factor signaling

✓ *EGFR*

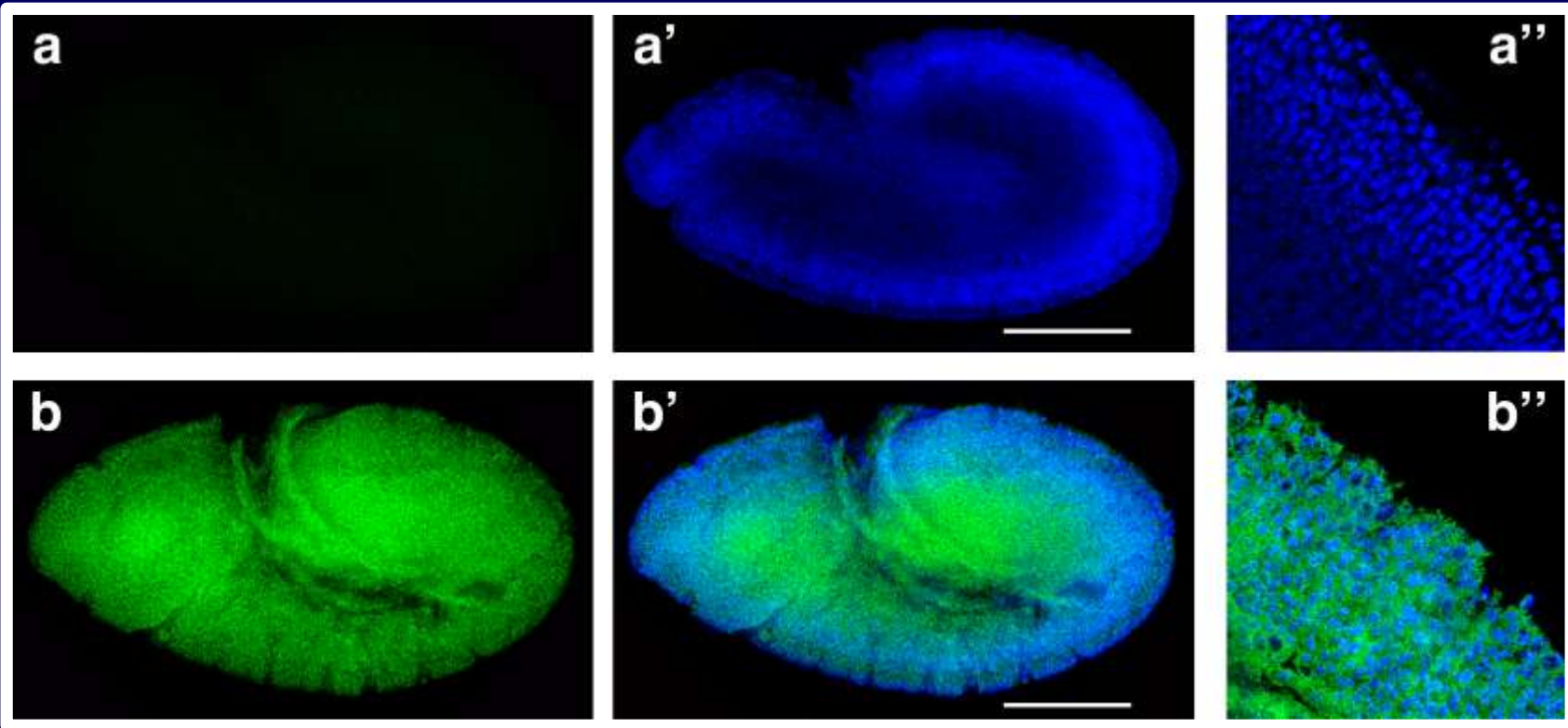
Myogenesis: from precursor cell to syncytial muscle



- Hypothesis- Human PAX-FKHR drives myogenesis in fly tissues
- Experiment:
 - Ubiquitous expression (*daughterless-Gal4* >> *UAS-PAX-FKHR*) of PAX-FKHR during embryogenesis
 - Test for MHC expression (**MHC-GFP reporter**)

Human PAX-FKHR Drives Myogenesis in *Drosophila* Embryos

*wild-type,
MHC-GFP*



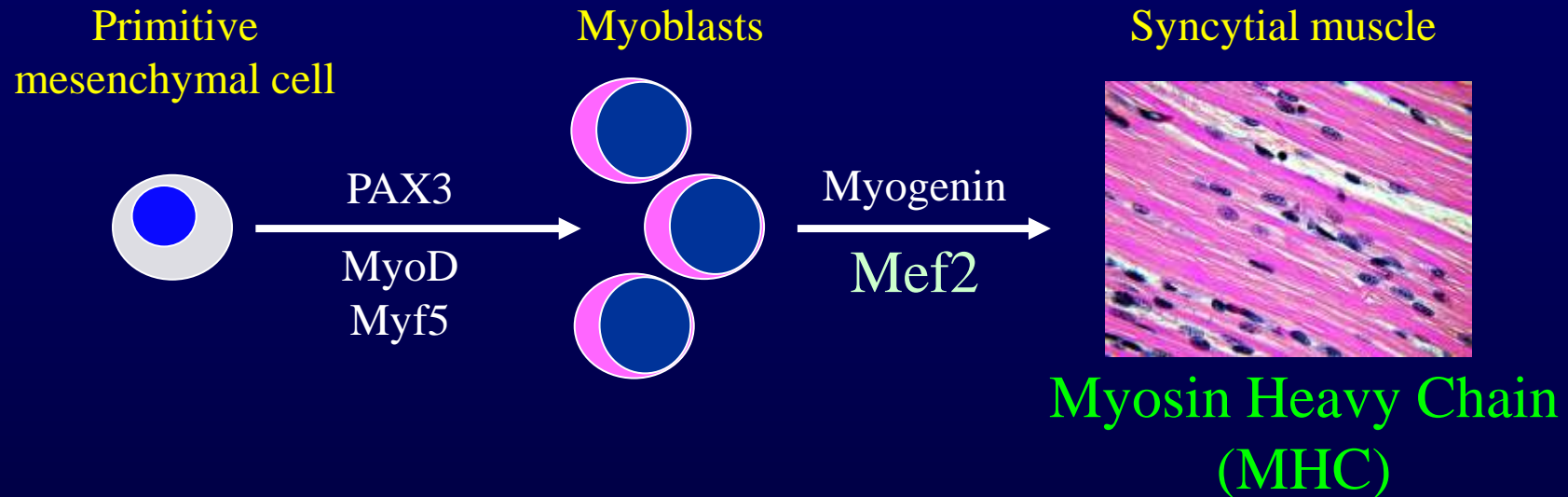
*PAX-FKHR,
MHC-GFP*

PAX-FKHR=daughterless-Gal4; UAS-PAX-FKHR

Blue=DAPI

Green=anti-GFP immunofluorescence

Myogenesis: from precursor cell to syncytial muscle



- *Mef2* is a critical gene in the myogenesis transcription factor cascade, and the master regulator of myogenesis in flies
- Hypotheses:
 - PAX-FKHR drives expression of *D-Mef2*
 - *D-Mef2* mutation will suppress PAX-FKHR pathogenicity

PAX-FKHR Screening and *D-Mef2*

9577;96A17-18	2363	0	194	194	0%
9577;D11;95F15	4332	0	225	225	0%
91A-8A102	2277	0	115	115	0%
75A6-7;75C1-2	2608	0	217	217	0%
646C;65C	3006	0	54	54	0%
75H10-11;76A1-5	6734	0	77	77	0%
569B-13;70A1-12	3467	1	74	74	1%
85A2;85C1-2	1962	3	189	192	2%
70C1-3;70D4-5	3124	1	50	51	2%
49C1-4;50C23-D2	442	2	88	90	2%
60E2-3;60E11-12	2471	5	140	145	3%
71F1-4;72D1-10	1075	5	124	129	4%
63F6-7;64C13-15	3688	4	97	101	4%
84A1-9;85E1-4	2268	1	71	72	4%
49A4-11;49E7-F1	724	6	128	134	4%
93B6-7;93D2	1340	6	38	44	3%
5702-6;58D1	5246	8	140	148	3%
88E7-11;89A1	183	13	175	188	7%
84B2;84B17	3096	5	64	71	7%
81F3-6;82F5-7	1518	9	117	126	8%
60C8;60E8	9005	0	312	312	0%
53D9-E1;54B5-10	7445	12	112	124	10%
54C1-4;54C14	6574	10	109	119	10%
85B9-12;85E7-F1	1671	18	125	143	10%
97E3;98A3	1823	18	152	170	10%
41A1;41A	739	22	167	189	12%
60F1;60F5	4915	11	89	99	12%
66B9-9;66C9-10	1541	19	129	148	13%
63B1-6;64B-9	463	42	276	318	17%
84A5;84D9	1842	18	106	124	15%
82F8-10;83A1-3	5694	34	196	230	15%
62A10-18;62D2-5	600	14	80	94	15%
96A1;96F;D	9500	17	93	110	15%
77E1;77F;78A	3127	35	189	225	16%
59D5-10;60B3-8	1882	18	94	112	16%
44D1-4;44F12	201	38	196	234	16%
56F12-14;57A4	6609	14	71	85	16%
99E5-4;99F1	2547	51	264	315	17%
95D8;95E5	7676	22	109	131	17%
95D3;95I	2546	41	107	148	17%
37E	447	17	81	98	17%
53E4;53F6	7876	29	136	165	18%
96C9;96E2	7981	32	150	182	18%
77F3;77E-9	4429	83	478	561	18%
67E3-7;68A2-6	6471	21	287	308	17%
48A3-4;8C6-8	1145	22	97	119	18%
96A1;97B1	5601	28	123	151	19%
84A1-2;84B1-2	1884	26	114	140	19%
83B7-C1;83E6-D1	7443	32	140	172	19%
51D3-8;52F5-9	3518	27	118	145	19%
78D3;78A2	8101	93	390	483	19%
42A1-2;42E6-F1	1007	28	116	144	19%
83E1-2;84A5	2393	19	78	97	20%
71E1-4;72D1-10	3640	98	390	488	20%
87E1-2;98A	9529	46	176	222	21%
62A2;62A7	6786	103	389	492	21%
54B17-C;54C1-4	5680	34	126	160	21%
77E2-4;78A2-4	3878	44	163	207	21%
73A3;74F	2998	39	134	173	21%
96A2;96A6	7882	37	160	194	23%
88E3;99A6-8	430	63	210	273	23%
95A1-4;95A8-B1	9497	43	141	184	23%
94E9;94E13	7990	37	118	155	24%
95C12;95D8	5279	52	153	207	25%
54B1-3;54B7-10	444	47	218	292	25%
60C5-6;60D9-10	2664	39	109	148	26%
66A17-10;66C1-5	5877	23	62	85	27%
68C8-11;69B4-5	2612	47	126	173	27%
88C1-8;81E1-5	1128	38	99	137	28%
77A1;77D1	2082	58	150	208	28%
77A1;77D1	8882	35	90	125	28%
74E7;74A1	787	31	79	110	28%
69A4-5;69D4-6	5492	43	107	150	29%
85B1-2;85A	282	40	92	132	30%
83A4;83B6	8103	50	104	154	32%
85A;85F	1547	71	147	218	33%
69E1;95D1	7992	53	109	162	33%
69E6;70A1;70A1-2	6457	36	74	110	33%
88E3;99A6-8	430	38	78	116	33%
45E4;46E12	8410	43	86	129	33%
59A1-3;59D1-4	3909	43	85	128	34%
87A;88A1-2	1910	90	176	266	34%
65F1;66D1	1420	41	79	120	34%
54C1-5;60B10	6866	48	88	126	37%
72C1-D1;73A3-4	2993	58	106	164	35%
88E3;99A6-8	430	39	70	109	36%
62B4-C;62D5-E5	2460	51	89	140	36%
46A;46E	1743	50	86	136	37%
84D1-2;84E5-7	6779	58	99	157	37%
45A6-7;45E2-3	4966	83	140	223	37%
55E11;55E16	7896	56	94	150	37%
42B3-5;43E15-18	1888	56	91	147	38%
78E4;78B	5126	42	67	109	39%
74D3-7;8A1;75B2-5	6411	53	84	137	39%
68A3-5;69A1-3	6511	38	60	98	39%
48C5-D1;48D5-E1	7148	87	109	169	40%
48E1;49A	4966	56	85	141	40%
82F3-4;82F10-11	4787	59	87	146	40%
83F9;84E1-2	8799	41	59	100	41%
85C4-4;85D12-14	1756	61	85	146	42%
86D7-13;86F9-12	6647	67	92	159	42%
66E1-2;67B3-3	7079	102	262	464	42%
60B9;60C4	9691	76	103	179	42%
76A7-8;1;76B4-5	198	61	81	142	43%
88B1-2;88B3-5	7412	129	169	298	43%
44E10;45D1-E1	3201	46	60	106	43%
66D10-11;66E1-2	3024	51	63	114	45%
67A2;67D11-13	967	47	58	105	45%
95A4;95B1	7674	45	55	100	45%
59E;59D8-11	7273	59	72	131	46%
50D1;50D7	6516	101	120	221	46%
49D3-4;49F6-6	6017	64	75	139	46%
50D4;50D4	7875	105	120	225	47%
95A5-7;95D6-11	7889	99	111	212	47%
48E12-14;48A11;86	5879	79	88	167	47%
84C8-D1;84E2-7	7441	98	109	207	47%
42E;44C	3268	59	64	123	48%
69E4;69F9-7	6535	62	67	129	48%
66E1-6;66F1-6	4900	43	45	88	49%
71C2-3;72B1-C1	6531	67	67	134	50%
78E1-F1;80A2-3	6681	72	71	151	51%
95A5-7;95C10-11	4940	88	92	110	53%
86E;86H	1702	84	72	156	54%
80E-F1;81E2-4	6855	47	35	81	59%

- *Df(2R)X1* is a suppressor

- *Dmef2²²⁻²¹* (null allele) strongly suppresses PAX-FKHR

- Microarray analysis of *wild-type* versus *PAX-FKHR* muscle: ~750 genes differentially expressed

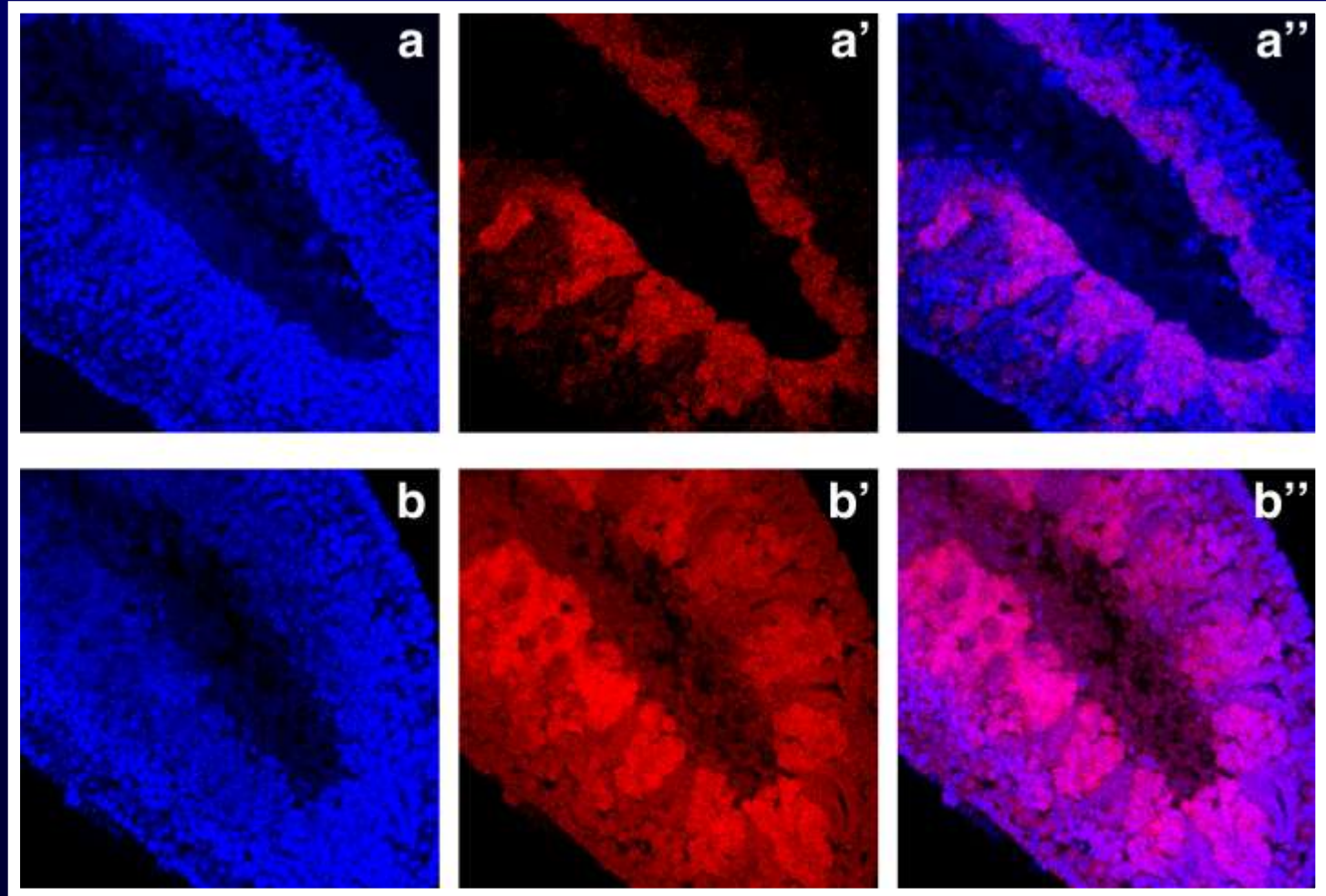
- Misexpressed genes = gene targets

- Unaltered genes potential co-factors

- Other myogenesis regulators- e.g, *MyoD*, *muscleblind*

D-Mef2 is a PAX-FKHR Target Gene in *Drosophila* Embryos

*wild-type,
D-Mef2-GFP*

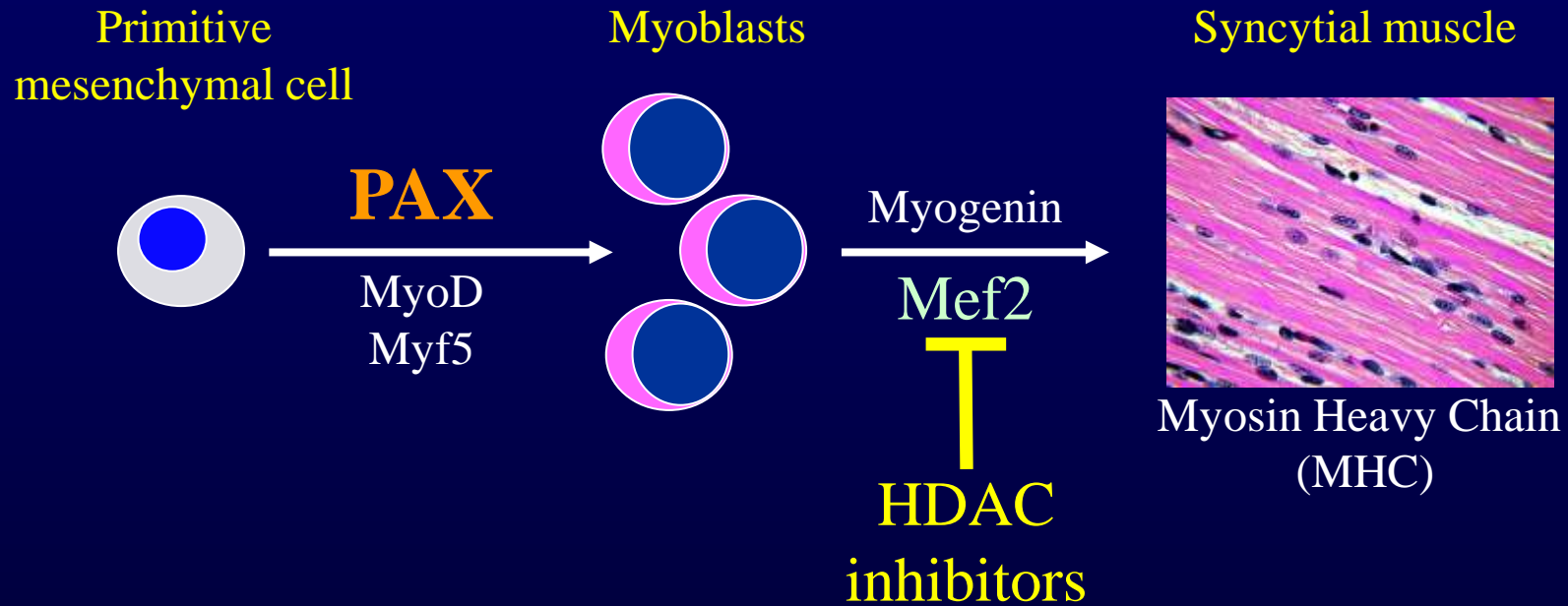


*PAX-FKHR,
D-Mef2-GFP*

PAX-FKHR=*daughterless-Gal4*; *UAS-PAX-FKHR*

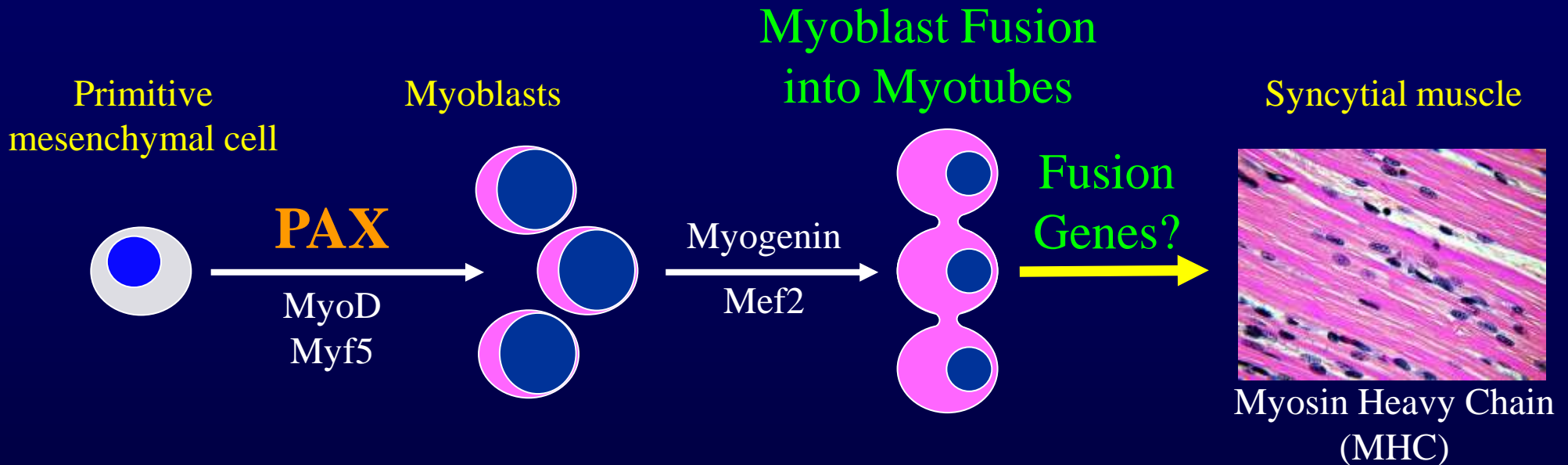
Blue=DAPI, Red=anti-GFP immunofluorescence

Myogenesis: from precursor cell to syncytial muscle



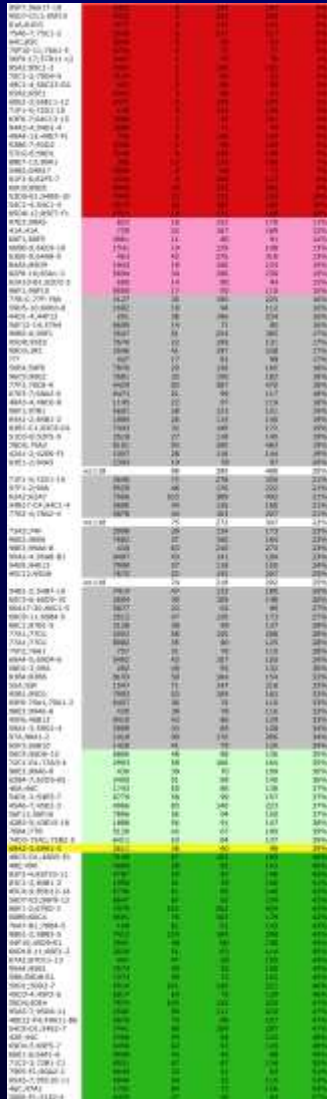
- *Mef2* involvement in RMS has not been reported
- “Gotta love the fly”- ONE *Mef2* gene versus FOUR in mammals!
- Drugs that “poison” *Mef2* activity (HDAC inhibitors) are new potential RMS therapies

Myogenesis: from precursor cell to syncytial muscle



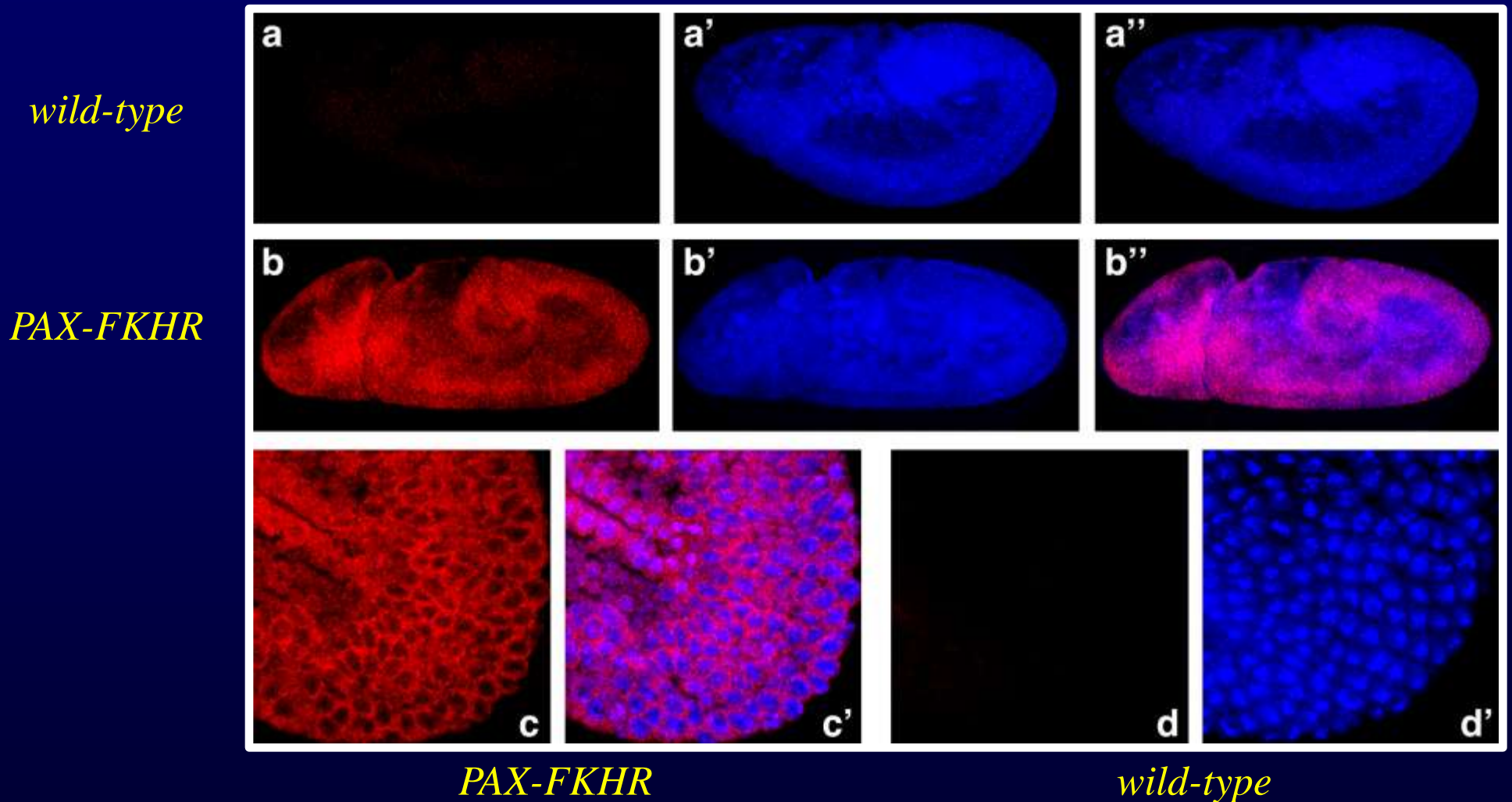
- Myoblast fusion genes are required to form syncytial tissue
- Little is known regarding mammalian myoblast fusion genes
- Could fusion gene misregulation underlie RMS pathogenesis?

PAX-FKHR Screening and Results



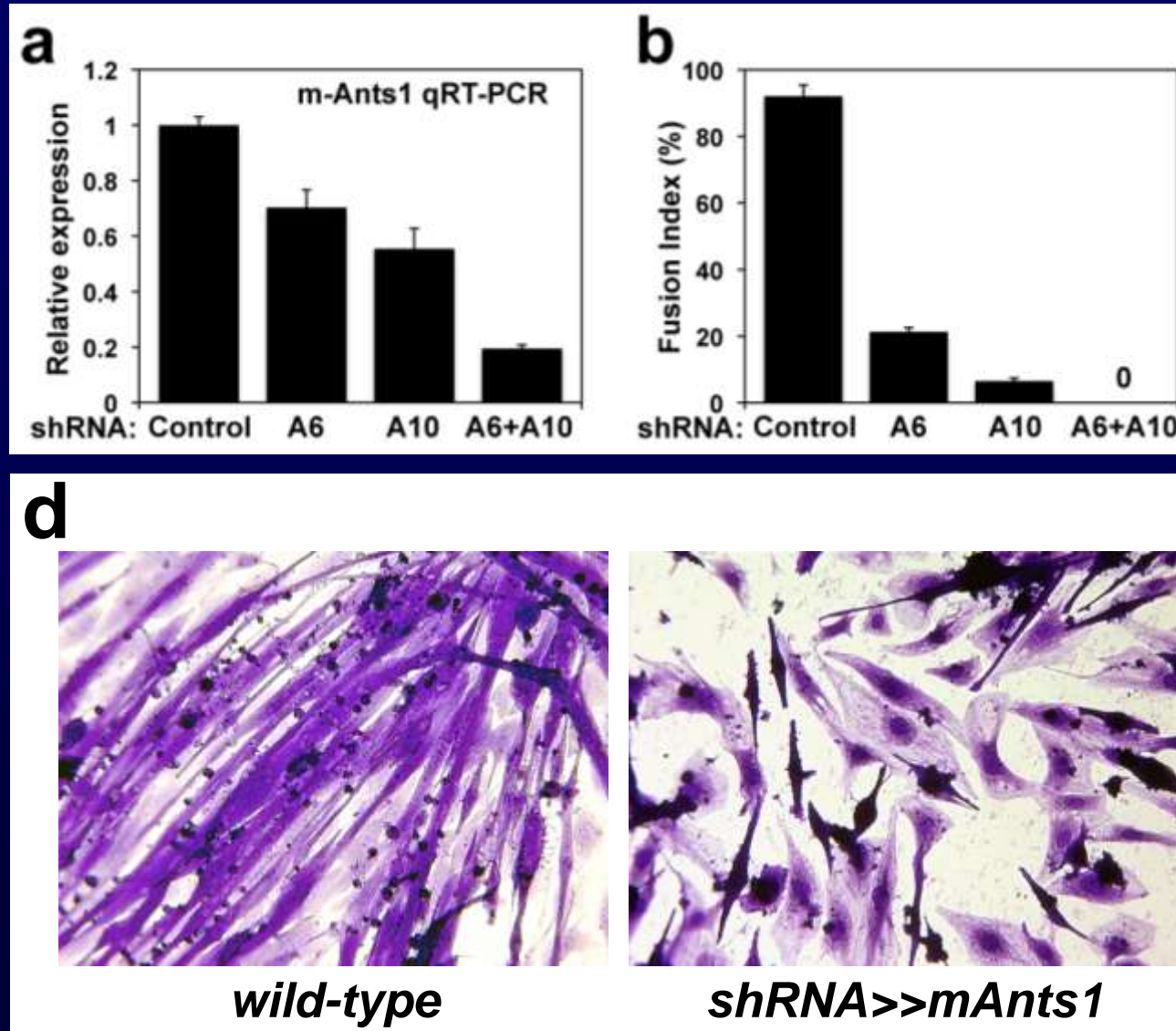
- *Df(3L)vin5* is a strong suppressor
- *antisocial (ants)^{P1729} (rols) LOF allele* strongly suppresses PAX-FKHR
- Microarray analysis of wild-type versus PAX-FKHR muscle: ~750 genes differentially expressed
 - Misexpressed genes = gene targets
 - Unaltered genes potential co-factors
- Other myoblast fusion regulators- e.g., *blown-fuse*, *myoblast city*

ants is a PAX-FKHR Target Gene



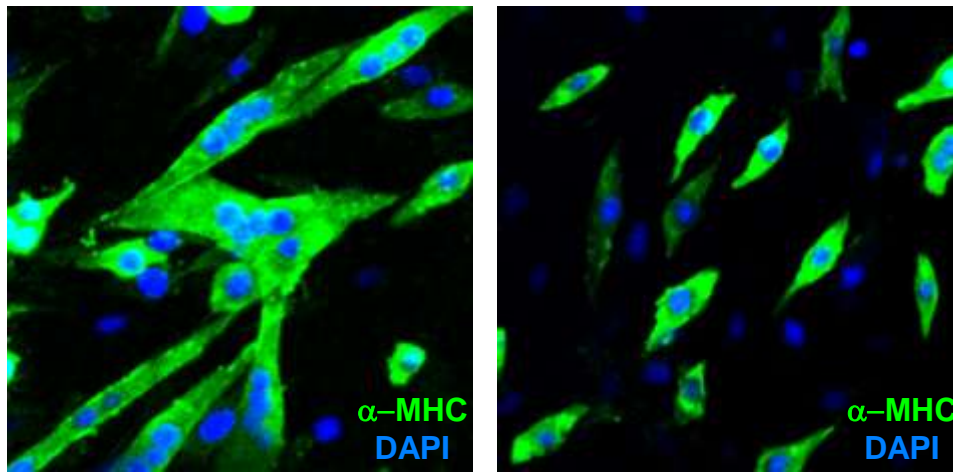
PAX-FKHR=*daughterless-Gal4*; *UAS-PAX-FKHR* | Blue=DAPI | Red=anti-Ants immunofluorescence

Ants1 (Tanc1) is a Mammalian Myoblast Fusion Gene



Ant1 is Not Required for Differentiation

B

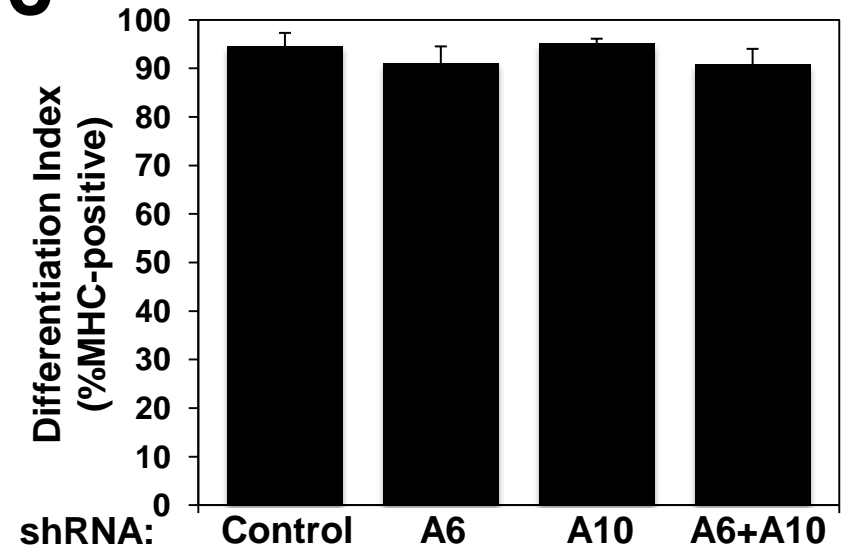


wild-type

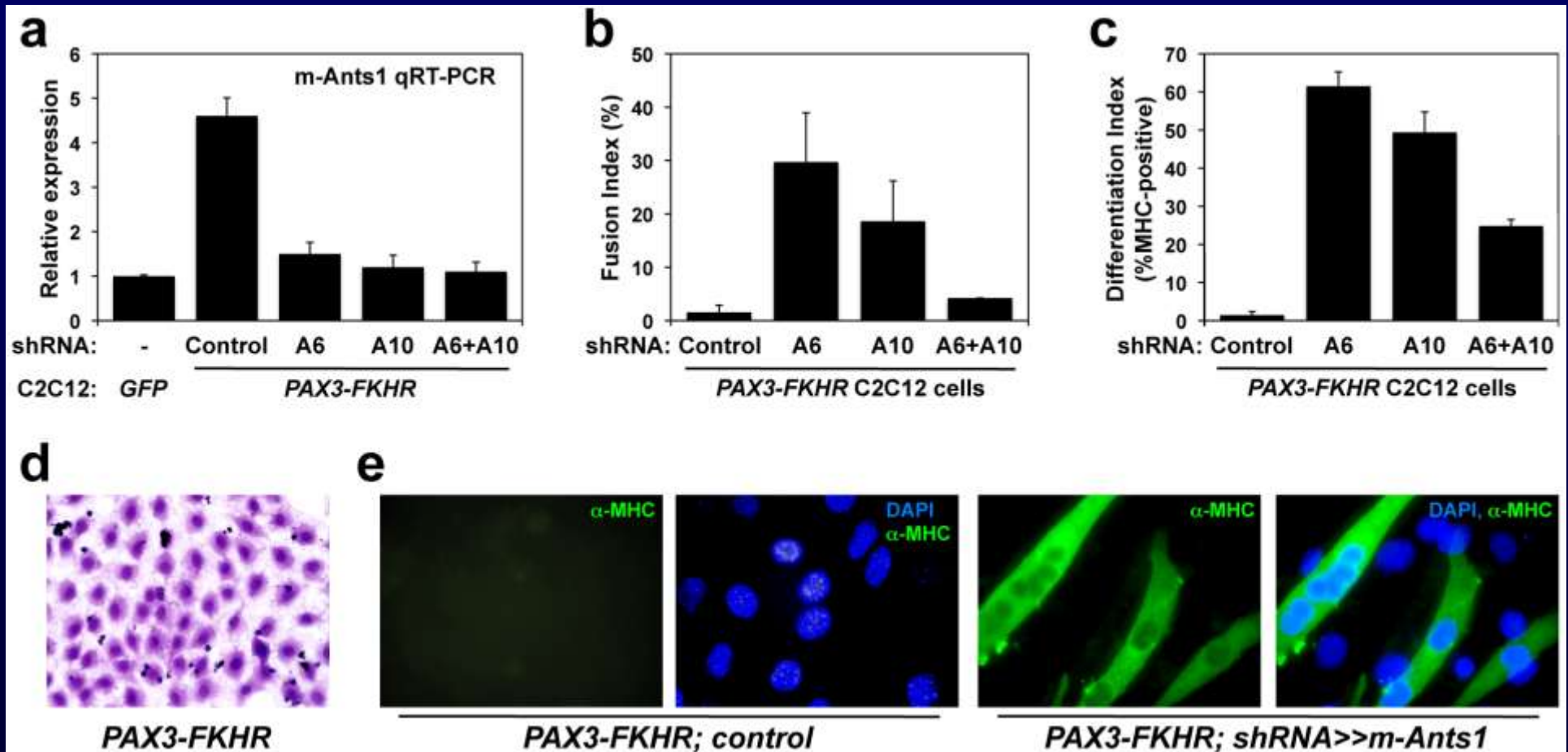
shRNA>>mAnts1

anti-Myosin Heavy Chain (MHC)

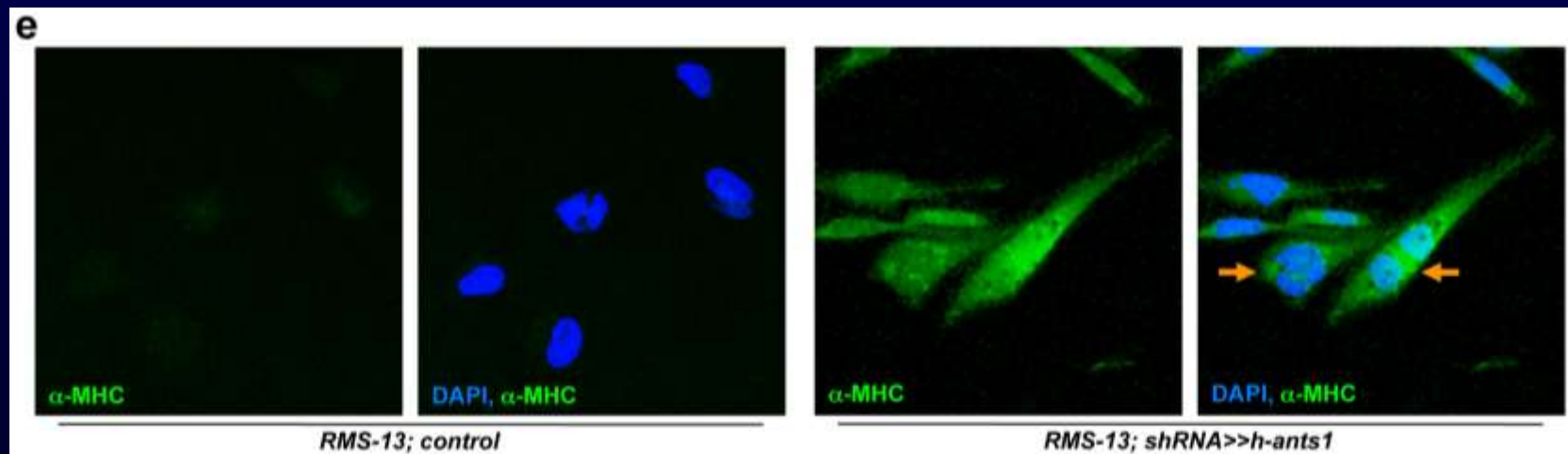
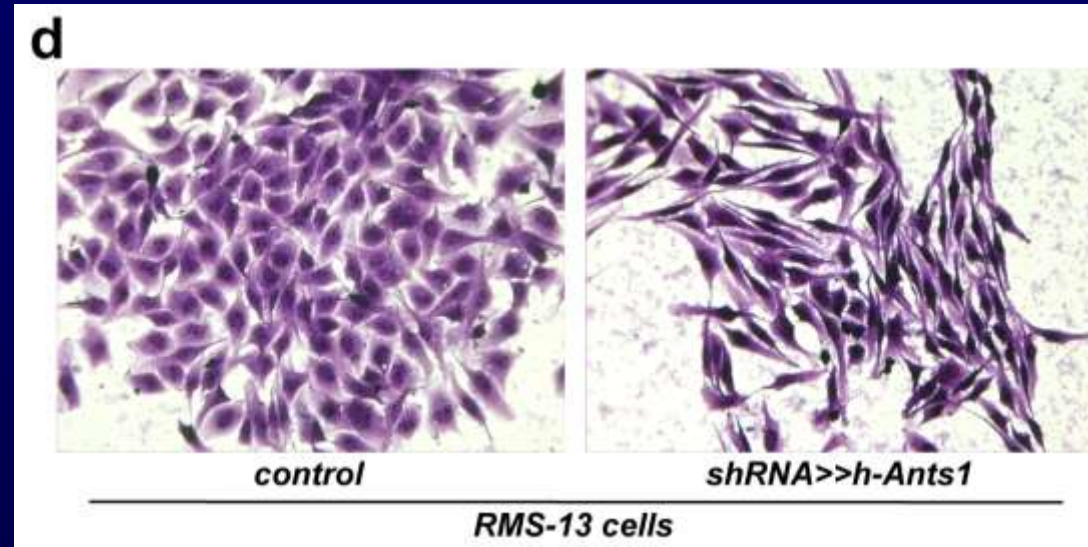
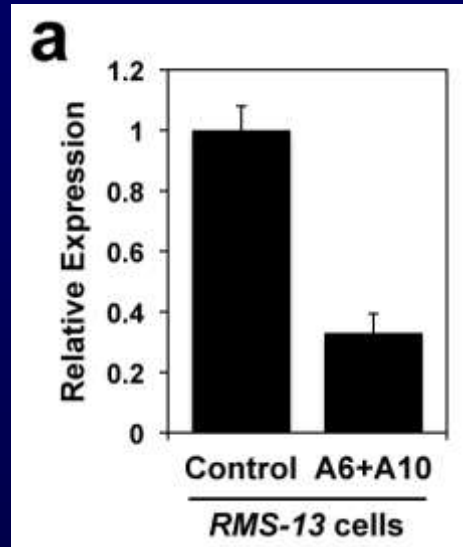
C



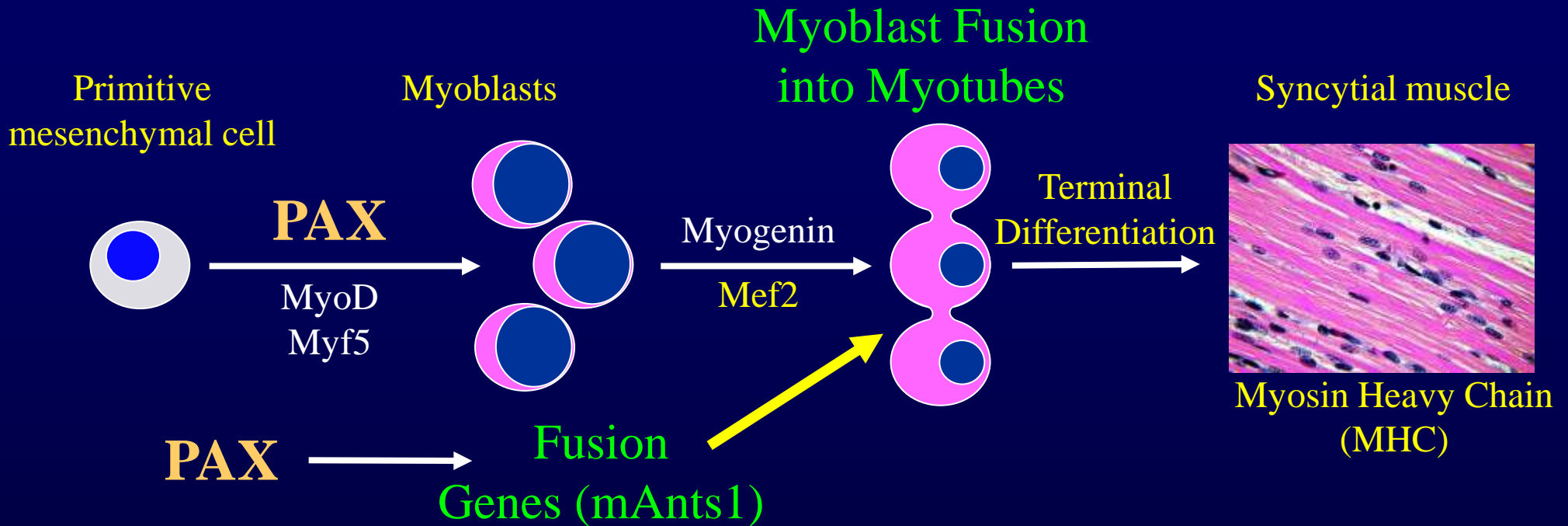
Ants1-Silencing Suppresses PAX-FKHR Pathogenicity in Mammalian Myoblasts



Ants1-Silencing Rescues PAX-FKHR RMS Cells from Differentiation-Arrest and Fusion Failure



Myogenesis, RMS Pathogenesis, and *Ants1*



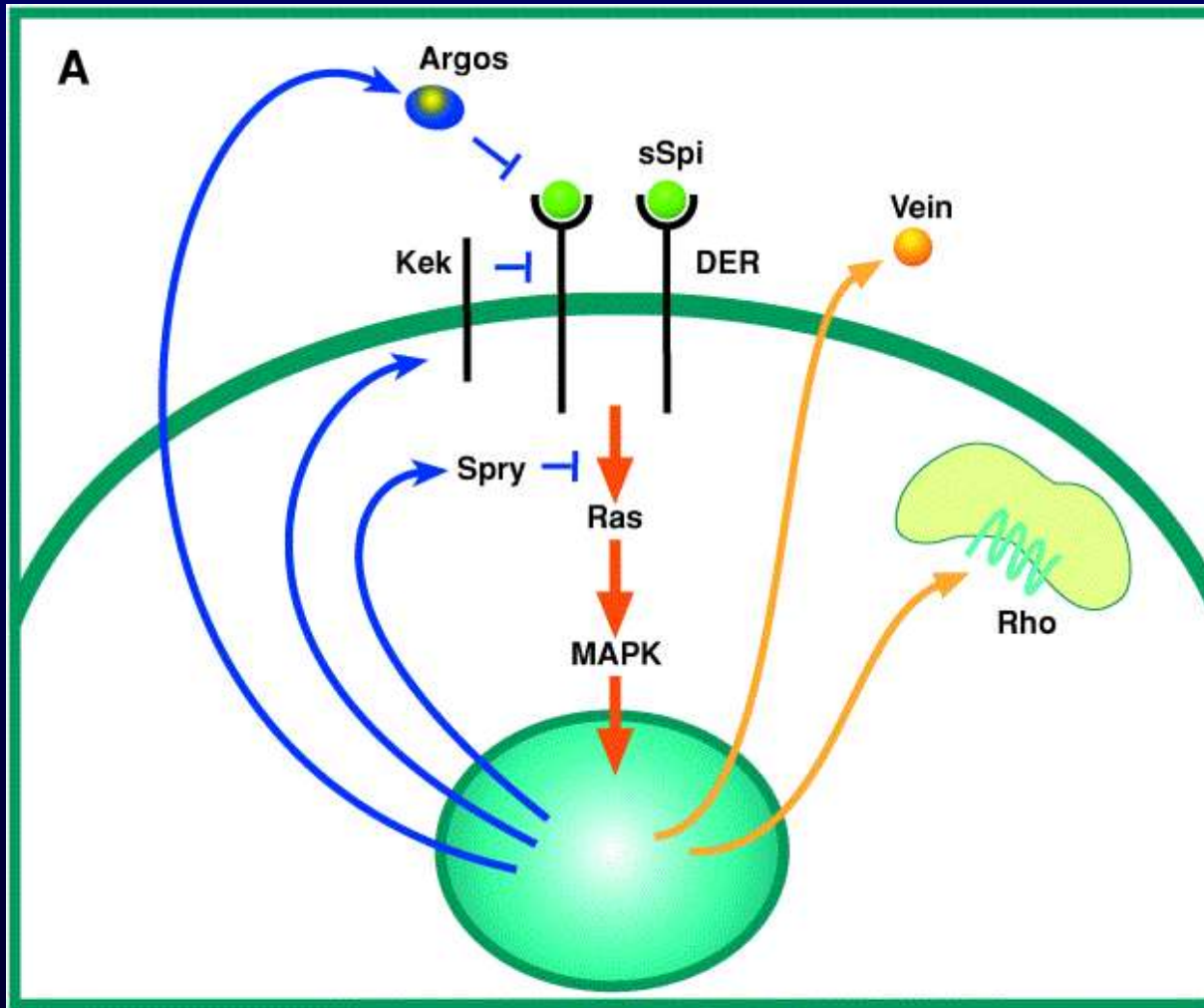
- *Ants1* is a PAX-FKHR target and mammalian myoblast fusion gene
- Correction of *Ants1* expression rescues RMS terminal differentiation arrest; mAnts is a new putative RMS disease gene
- *In Vivo* profiling: xenografts, gene sequencing, CGH

PAX-FKHR Screening and Results

Gene	FC	FC	FC	FC
ADAM10	1.2	1.1	1.1	1.1
ADAM10-AS1	1.2	1.1	1.1	1.1
ADAM10-AS2	1.2	1.1	1.1	1.1
ADAM10-AS3	1.2	1.1	1.1	1.1
ADAM10-AS4	1.2	1.1	1.1	1.1
ADAM10-AS5	1.2	1.1	1.1	1.1
ADAM10-AS6	1.2	1.1	1.1	1.1
ADAM10-AS7	1.2	1.1	1.1	1.1
ADAM10-AS8	1.2	1.1	1.1	1.1
ADAM10-AS9	1.2	1.1	1.1	1.1
ADAM10-AS10	1.2	1.1	1.1	1.1
ADAM10-AS11	1.2	1.1	1.1	1.1
ADAM10-AS12	1.2	1.1	1.1	1.1
ADAM10-AS13	1.2	1.1	1.1	1.1
ADAM10-AS14	1.2	1.1	1.1	1.1
ADAM10-AS15	1.2	1.1	1.1	1.1
ADAM10-AS16	1.2	1.1	1.1	1.1
ADAM10-AS17	1.2	1.1	1.1	1.1
ADAM10-AS18	1.2	1.1	1.1	1.1
ADAM10-AS19	1.2	1.1	1.1	1.1
ADAM10-AS20	1.2	1.1	1.1	1.1
ADAM10-AS21	1.2	1.1	1.1	1.1
ADAM10-AS22	1.2	1.1	1.1	1.1
ADAM10-AS23	1.2	1.1	1.1	1.1
ADAM10-AS24	1.2	1.1	1.1	1.1
ADAM10-AS25	1.2	1.1	1.1	1.1
ADAM10-AS26	1.2	1.1	1.1	1.1
ADAM10-AS27	1.2	1.1	1.1	1.1
ADAM10-AS28	1.2	1.1	1.1	1.1
ADAM10-AS29	1.2	1.1	1.1	1.1
ADAM10-AS30	1.2	1.1	1.1	1.1
ADAM10-AS31	1.2	1.1	1.1	1.1
ADAM10-AS32	1.2	1.1	1.1	1.1
ADAM10-AS33	1.2	1.1	1.1	1.1
ADAM10-AS34	1.2	1.1	1.1	1.1
ADAM10-AS35	1.2	1.1	1.1	1.1
ADAM10-AS36	1.2	1.1	1.1	1.1
ADAM10-AS37	1.2	1.1	1.1	1.1
ADAM10-AS38	1.2	1.1	1.1	1.1
ADAM10-AS39	1.2	1.1	1.1	1.1
ADAM10-AS40	1.2	1.1	1.1	1.1
ADAM10-AS41	1.2	1.1	1.1	1.1
ADAM10-AS42	1.2	1.1	1.1	1.1
ADAM10-AS43	1.2	1.1	1.1	1.1
ADAM10-AS44	1.2	1.1	1.1	1.1
ADAM10-AS45	1.2	1.1	1.1	1.1
ADAM10-AS46	1.2	1.1	1.1	1.1
ADAM10-AS47	1.2	1.1	1.1	1.1
ADAM10-AS48	1.2	1.1	1.1	1.1
ADAM10-AS49	1.2	1.1	1.1	1.1
ADAM10-AS50	1.2	1.1	1.1	1.1
ADAM10-AS51	1.2	1.1	1.1	1.1
ADAM10-AS52	1.2	1.1	1.1	1.1
ADAM10-AS53	1.2	1.1	1.1	1.1
ADAM10-AS54	1.2	1.1	1.1	1.1
ADAM10-AS55	1.2	1.1	1.1	1.1
ADAM10-AS56	1.2	1.1	1.1	1.1
ADAM10-AS57	1.2	1.1	1.1	1.1
ADAM10-AS58	1.2	1.1	1.1	1.1
ADAM10-AS59	1.2	1.1	1.1	1.1
ADAM10-AS60	1.2	1.1	1.1	1.1
ADAM10-AS61	1.2	1.1	1.1	1.1
ADAM10-AS62	1.2	1.1	1.1	1.1
ADAM10-AS63	1.2	1.1	1.1	1.1
ADAM10-AS64	1.2	1.1	1.1	1.1
ADAM10-AS65	1.2	1.1	1.1	1.1
ADAM10-AS66	1.2	1.1	1.1	1.1
ADAM10-AS67	1.2	1.1	1.1	1.1
ADAM10-AS68	1.2	1.1	1.1	1.1
ADAM10-AS69	1.2	1.1	1.1	1.1
ADAM10-AS70	1.2	1.1	1.1	1.1
ADAM10-AS71	1.2	1.1	1.1	1.1
ADAM10-AS72	1.2	1.1	1.1	1.1
ADAM10-AS73	1.2	1.1	1.1	1.1
ADAM10-AS74	1.2	1.1	1.1	1.1
ADAM10-AS75	1.2	1.1	1.1	1.1
ADAM10-AS76	1.2	1.1	1.1	1.1
ADAM10-AS77	1.2	1.1	1.1	1.1
ADAM10-AS78	1.2	1.1	1.1	1.1
ADAM10-AS79	1.2	1.1	1.1	1.1
ADAM10-AS80	1.2	1.1	1.1	1.1
ADAM10-AS81	1.2	1.1	1.1	1.1
ADAM10-AS82	1.2	1.1	1.1	1.1
ADAM10-AS83	1.2	1.1	1.1	1.1
ADAM10-AS84	1.2	1.1	1.1	1.1
ADAM10-AS85	1.2	1.1	1.1	1.1
ADAM10-AS86	1.2	1.1	1.1	1.1
ADAM10-AS87	1.2	1.1	1.1	1.1
ADAM10-AS88	1.2	1.1	1.1	1.1
ADAM10-AS89	1.2	1.1	1.1	1.1
ADAM10-AS90	1.2	1.1	1.1	1.1
ADAM10-AS91	1.2	1.1	1.1	1.1
ADAM10-AS92	1.2	1.1	1.1	1.1
ADAM10-AS93	1.2	1.1	1.1	1.1
ADAM10-AS94	1.2	1.1	1.1	1.1
ADAM10-AS95	1.2	1.1	1.1	1.1
ADAM10-AS96	1.2	1.1	1.1	1.1
ADAM10-AS97	1.2	1.1	1.1	1.1
ADAM10-AS98	1.2	1.1	1.1	1.1
ADAM10-AS99	1.2	1.1	1.1	1.1
ADAM10-AS100	1.2	1.1	1.1	1.1

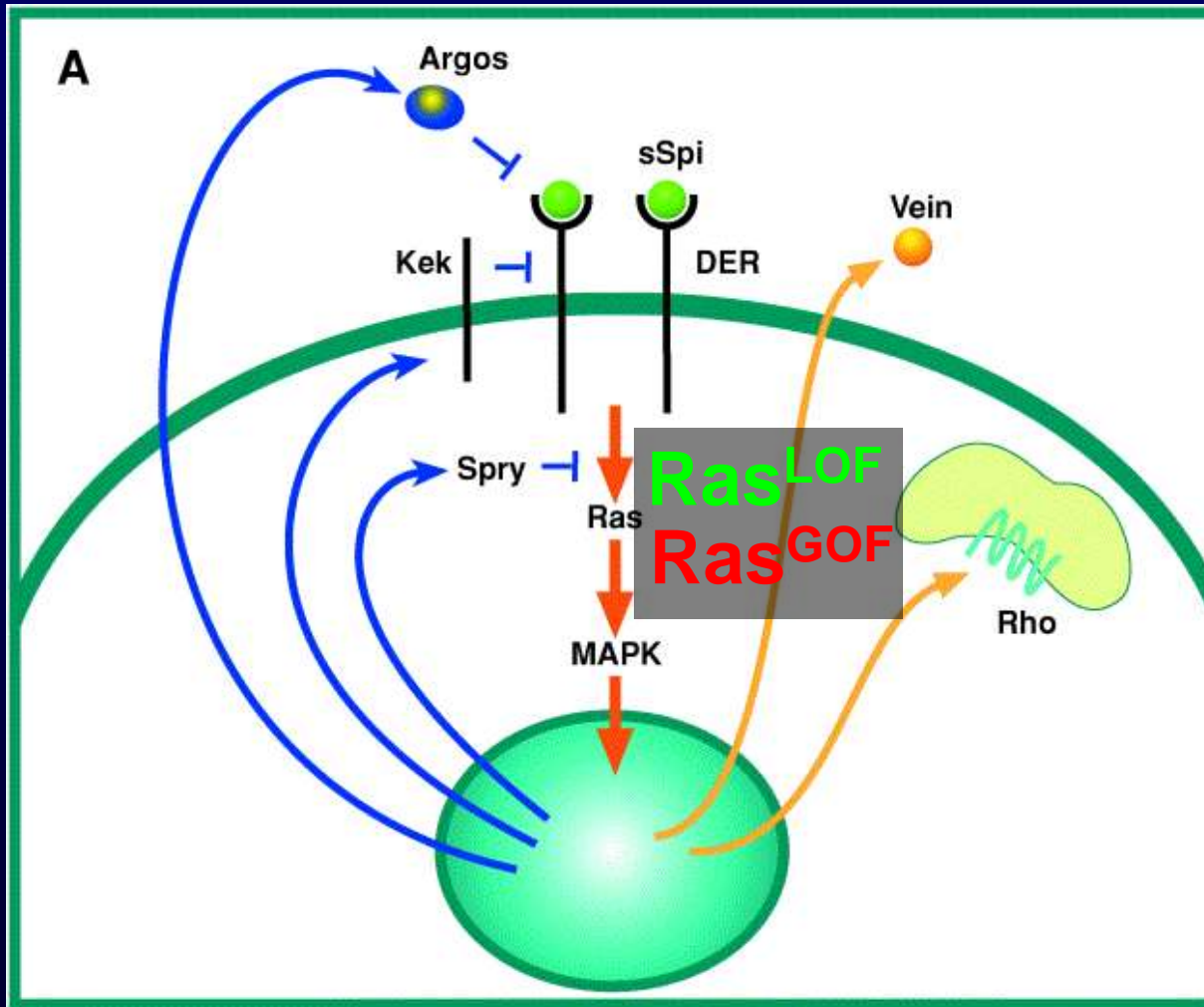
- Ras is a PAX-FKHR genetic interactor
- Microarray analysis of wild-type versus PAX-FKHR muscle: ~750 genes differentially expressed
 - Misexpressed genes = gene targets
 - Unaltered genes = potential co-factors
- Regulators of Epidermal Growth Factor signaling (*i.e.*, *EGFR* ligand, *EGFR* inhibitor, *EGFR*)

Epidermal Growth Factor Receptor Signaling and PAX-FKHR



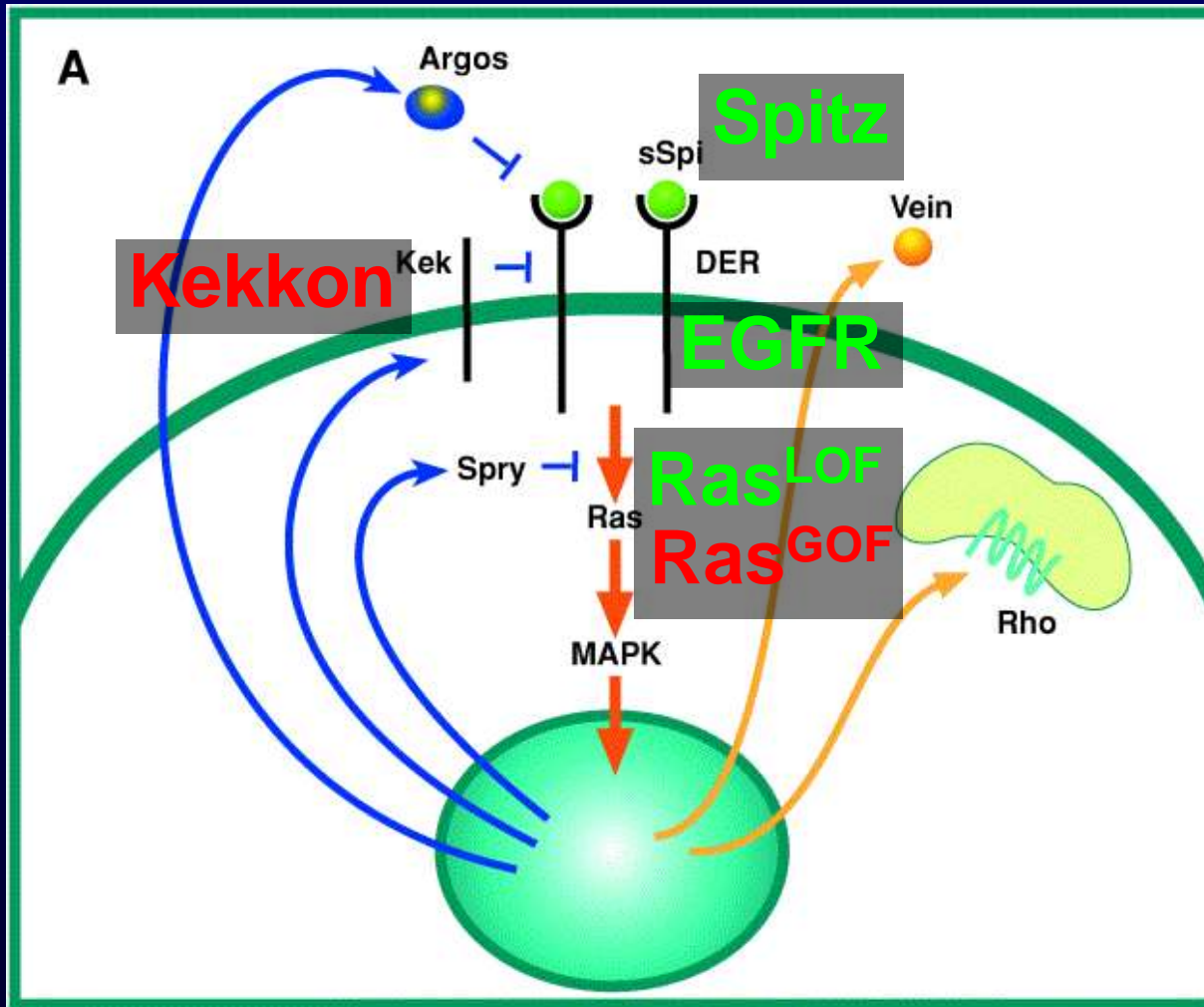
- ✓ DER = EGF Receptor
- ✓ sSpi = EGFR Ligand
- ✓ Ras- intracellular effector
- ✓ Kek = Kekkone; EGFR inhibitor

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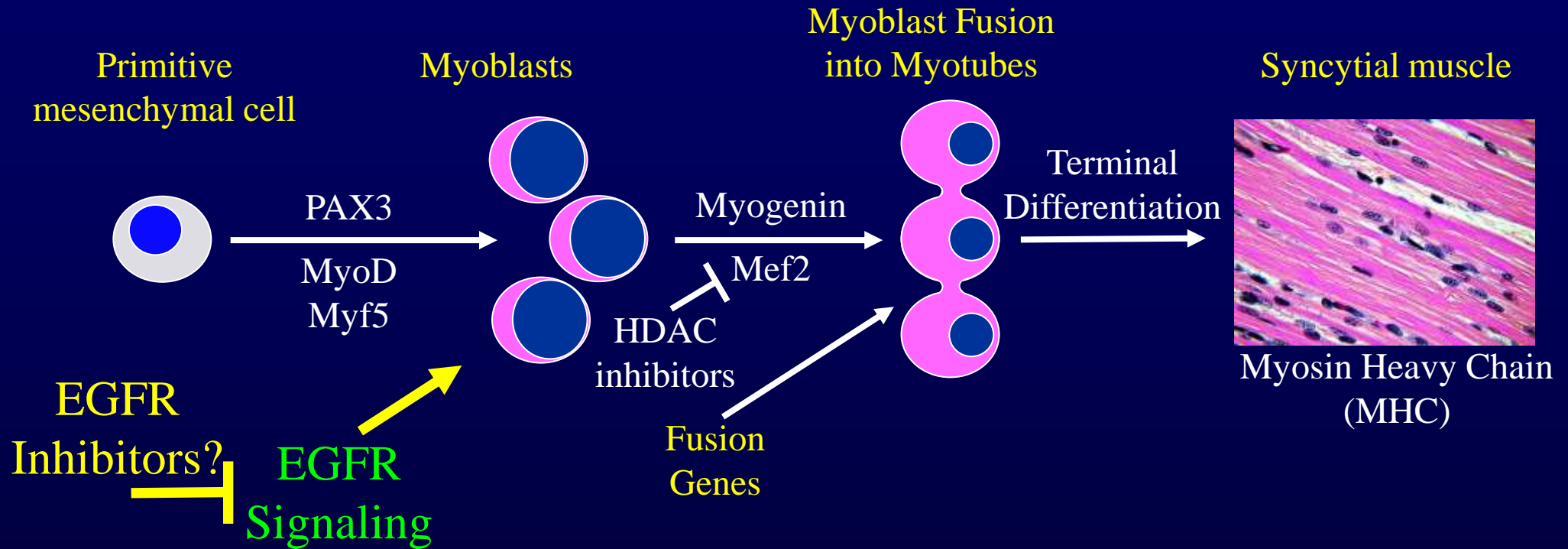
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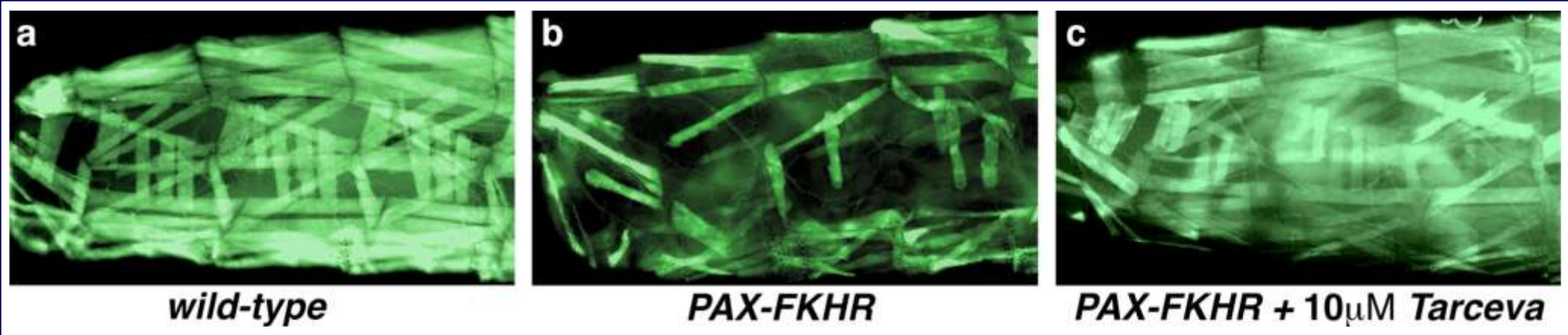
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Myogenesis: EGFR Signaling



- EGFR signaling participates in PAX-FKHR pathogenic activity
- Suggests that directed therapy targeting EGFR (Tarceva) might be a new, effective RMS therapy

Tarceva Therapy Blocks PAX-FKHR Pathogenicity *in vivo*



Summary and Future Studies

- Utilizing a *Drosophila* model system to identify new PAX-FKHR co-factors and gene targets
 - Genetic screening
 - microarray analysis
- Isolating:
 - Muscle development regulators: *Mef2*
 - Growth factor signaling: *EGFR*
 - Myoblast fusion elements: *Antisocial*

Summary and Future Studies

- Characterize PAX-FKHR co-factors/gene targets for activity in mammalian systems
 - Mammalian myoblasts
 - Tumor tissue/cell lines: *Gene Sequencing and Protein Expression*
 - Mouse RMS transgenic model
- Long term goals:
 - Drug screening
 - Tumor diagnosis and prognosis
 - General themes in pediatric sarcoma tumorigenesis?

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