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Supplementary Materials for

Skeletal muscle action of estrogen receptor α is critical for the maintenance of mitochondrial function and metabolic homeostasis in females

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The PDF file includes:

table S1. Basal metabolic characteristics in female subjects. Age, body weight, fat mass, body mass index, and glucose and insulin concentrations in healthy women (Healthy Cohort n=18; n=9 premenopausal and n=9 postmenopausal) and women displaying clinical features of the metabolic syndrome (MetSyn, n=25; n=21 premenopausal and n=4 postmenopausal). The healthy cohort of women were stratified by menopausal status (n=9/group) and age, body weight, BMI, fat free mass, fasting blood glucose, fasting insulin, estradiol, FSH, and LH values are provided in the lower panel. Values are expressed as means ± SD. Mean differences were detected by Student's t-test, and *P* values are provided for each comparison.

	Healthy	MetSyn	*P*
n	18	25	
Age studied (yrs)	46 ± 5	45 ± 2	0.934
Body weight (kg)	64 ± 2.0	89 ± 3.0	<0.001
Fat mass (kg)	24 ± 1	40 ± 2	<0.001
BMI	23 ± 1	33 ± 5	<0.001
Fasting blood glucose (mg/dl)	87 ± 2	90 ± 2	0.371
Fasting insulin (pmol/l)	41.6 ± 0.7	87.8 ± 7.9	<0.001

Healthy Cohort	*Premenopausal*	*Postmenopausal*	
n	9	9	
Age studied (yrs)	23 ± 0.5	68 ± 0.7	<0.001
Body weight (kg)	64 ± 2.6	65 ± 2.7	0.71
BMI	22 ± 0.79	24 ± 0.90	0.16
Fat free mass (kg)	40.6 ± 1.8	40.2 ± 0.87	0.84
Fasting blood glucose (mg/dl)	83 ± 3	94 ± 4	0.06
Fasting insulin (pmol/l)	43 ± 6.5	30.4 ± 4.0	0.11
Estradiol (pg/ml)	139 ± 40.5	14 ± 0.27	0.004
FSH (IU/L)	5.3 ± 0.51	68.9 ± 6.17	<0.001
LH (IU/L)	8.6 ± 1.38	28.2 ± 2.9	<0.001

table S2. Animal characteristics following a 6-hour fast in the basal or clamped state. Body weights and biochemical parameters are represented as mean ± SEM, *n*=6-16 female animals, per genotype (Control f/f vs. MERKO), normal chow (NC) fed. Mean differences were detected by one-way ANOVA or Student's t-test where appropriate, and exact *P* values are provided for each comparison

	Control f/f	MERKO	*P*
n basal	16	16	
n clamp	7	7	
n muscle glucose uptake	6	6	
Age studied (wk)	12-28	12-28	
<u>Circulating hormones and adipokines</u>			
Fasting insulin (ng/ml), 12 weeks	0.22 ± 0.02	0.40 ± 0.03	0.01
Adiponectin (μg/ml), 12 weeks	44 ± 1.4	29 ± 0.08	0.004
Leptin (ng/ml), 12 weeks	1.5 ± 0.3	3.0 ± 0.4	0.001
<u>Body weight (g)</u>			
Age 10-12 weeks	21.3 ± 0.5	22.3 ± 0.7	0.286
Age 24 weeks (muscle GU studies)	23.7 ± 0.4	26.2 ± 1.1	0.008
Age 28 weeks (clamp studies)	24.4 ± 0.6	29.5 ± 1.4	0.004
<u>Tissue weights</u> <u>(g) 28 weeks of age</u>			
Gonadal fat	0.43 ± 0.08	1.08 ± 0.23	0.019
Liver weight	0.87 ± 0.02	0.98 ± 0.03	0.012
Heart weight	0.107 ± 0.004	0.114 ± 0.002	0.17
<u>Clamp studies</u>			
Fasting blood glucose (mg/dl)	124 ± 2	116 ± 2	0.07
Clamp blood glucose (mg/dl)	114 ± 4	115 ± 2	0.77
Clamp insulin (ng/ml)	9 ± 2.5	11.8 ± 2	0.41
Basal glucose turnover (mg/kg/min)	21 ± 2.1	26 ± 1.3	0.06
GIR (mg/kg/min)	57 ± 3.1	40 ± 3.0	0.001

table S3. **Annotation of functionally enriched terms from Illumina microarray analyses on quadriceps muscle from female Control f/f versus MERKO mice** (n=4-5 pooled samples of 3 muscles per genotype). Gene-functional annotation enrichment analysis to identify overrepresented biological terms associated with upregulated or downregulated genes in MERKO muscle microarray. Annotation categories from Gene Ontology Biological Process / Cellular Component, KEGG Pathways or Tissue_UP were used to identify statistically significant enrichment using DAVID bioinformatics resources.

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Upregulated genes - Functional annotation enriched terms

Gene Ontology - Biological Process	Count	%	Fold Enrichment	*P*-Value
GO:0014706~striated muscle tissue development	8	8.1	10.8	8.16E-06
GO:0060537~muscle tissue development	8	8.1	10.2	1.19E-05
GO:0044057~regulation of system process	9	9.1	7.7	1.79E-05
GO:0002026~regulation of the force of heart contraction	4	4.0	49.3	6.14E-05
GO:0007517~muscle organ development	8	8.1	7.8	6.50E-05
GO:0006937~regulation of muscle contraction	5	5.1	19.5	1.13E-04
GO:0043434~response to peptide hormone stimulus	6	6.1	10.8	2.15E-04
GO:0009725~response to hormone stimulus	7	7.1	7.3	3.52E-04
GO:0034097~response to cytokine stimulus	4	4.0	23.7	5.91E-04
GO:0008203~cholesterol metabolic process	5	5.1	12.5	6.38E-04
GO:0009719~response to endogenous stimulus	7	7.1	6.5	6.55E-04
GO:0051336~regulation of hydrolase activity	7	7.1	6.1	9.05E-04
GO:0016125~sterol metabolic process	5	5.1	11.3	9.45E-04
GO:0051258~protein polymerization	4	4.0	17.3	1.50E-03
GO:0055002~striated muscle cell development	4	4.0	14.6	2.48E-03
GO:0008016~regulation of heart contraction	4	4.0	13.3	3.18E-03
GO:0006869~lipid transport	5	5.1	7.8	3.58E-03
GO:0055001~muscle cell development	4	4.0	12.6	3.78E-03
GO:0048738~cardiac muscle tissue development	4	4.0	11.9	4.45E-03
GO:0010876~lipid localization	5	5.1	7.3	4.69E-03
GO:0042157~lipoprotein metabolic process	4	4.0	9.9	7.46E-03
GO:0051346~negative regulation of hydrolase activity	3	3.0	21.8	7.99E-03
GO:0007519~skeletal muscle tissue development	4	4.0	9.6	8.11E-03
GO:0060538~skeletal muscle organ development	4	4.0	9.3	8.80E-03
GO:0006163~purine nucleotide metabolic process	5	5.1	5.7	1.11E-02
GO:0051146~striated muscle cell differentiation	4	4.0	8.1	1.27E-02
GO:0010927~cellular component assembly involved in morphogenesis	3	3.0	16.6	1.37E-02
GO:0008202~steroid metabolic process	5	5.1	5.3	1.37E-02
GO:0048747~muscle fiber development	3	3.0	15.5	1.55E-02
GO:0006873~cellular ion homeostasis	6	6.1	4.0	1.61E-02

GO:0048878~chemical homeostasis	7	7.1	3.4	1.64E-02
GO:0055082~cellular chemical homeostasis	6	6.1	3.9	1.75E-02
GO:0043623~cellular protein complex assembly	4	4.0	6.7	2.13E-02
GO:0050801~ion homeostasis	6	6.1	3.6	2.43E-02
GO:0030300~regulation of intestinal cholesterol absorption	2	2.0	80.1	2.44E-02
GO:0010033~response to organic substance	8	8.1	2.7	2.46E-02
GO:0042692~muscle cell differentiation	4	4.0	6.0	2.76E-02
GO:0032371~regulation of sterol transport	2	2.0	64.1	3.04E-02
GO:0032374~regulation of cholesterol transport	2	2.0	64.1	3.04E-02
GO:0043462~regulation of ATPase activity	2	2.0	64.1	3.04E-02
GO:0019932~second-messenger-mediated signaling	4	4.0	5.8	3.10E-02
GO:0044093~positive regulation of molecular function	6	6.1	3.3	3.31E-02
GO:0032368~regulation of lipid transport	2	2.0	53.4	3.64E-02
GO:0044058~regulation of digestive system process	2	2.0	53.4	3.64E-02
GO:0006164~purine nucleotide biosynthetic process	4	4.0	5.3	3.86E-02
GO:0030005~cellular di-, tri-valent inorganic cation homeostasis	4	4.0	5.3	3.94E-02
GO:0044092~negative regulation of molecular function	4	4.0	5.1	4.18E-02
GO:0042592~homeostatic process	8	8.1	2.4	4.35E-02
GO:0019725~cellular homeostasis	6	6.1	3.1	4.41E-02
GO:0006936~muscle contraction	3	3.0	8.6	4.66E-02
GO:0045214~sarcomere organization	2	2.0	40.0	4.83E-02
GO:0055066~di-, tri-valent inorganic cation homeostasis	4	4.0	4.8	4.88E-02
Gene Ontology - Cell Component				
GO:0032994~protein-lipid complex	5	5.1	26.9	3.09E-05
GO:0034358~plasma lipoprotein particle	5	5.1	26.9	3.09E-05
GO:0030017~sarcomere	6	6.1	12.3	1.17E-04
GO:0044449~contractile fiber part	6	6.1	11.3	1.72E-04
GO:0030016~myofibril	6	6.1	10.7	2.19E-04
GO:0043292~contractile fiber	6	6.1	10.2	2.74E-04
GO:0005576~extracellular region	23	23.2	2.2	3.69E-04
GO:0005615~extracellular space	11	11.1	3.4	1.12E-03
GO:0034364~high-density lipoprotein particle	3	3.0	27.2	5.16E-03
GO:0044421~extracellular region part	12	12.1	2.5	7.93E-03
GO:0005759~mitochondrial matrix	5	5.1	4.8	1.99E-02
GO:0031980~mitochondrial lumen	5	5.1	4.8	1.99E-02
GO:0005856~cytoskeleton	13	13.1	2.0	2.88E-02
GO:0015629~actin cytoskeleton	5	5.1	4.2	3.07E-02
GO:0031674~I band	3	3.0	9.3	4.06E-02
GO:0044429~mitochondrial part	8	8.1	2.4	4.56E-02

GO:0005863~striated muscle thick filament	2	2.0	41.4	4.67E-02
KEGG Pathway				
mmu05414:Dilated cardiomyopathy	8	8.1	12.2	2.51E-06
mmu04260:Cardiac muscle contraction	6	6.1	11.2	1.50E-04
mmu04610:Complement and coagulation cascades	6	6.1	10.2	2.41E-04
mmu05410:Hypertrophic cardiomyopathy (HCM)	6	6.1	9.9	2.73E-04
mmu04540:Gap junction	5	5.1	7.9	3.11E-03
mmu03320:PPAR signaling pathway	4	4.0	6.7	2.01E-02
mmu04020:Calcium signaling pathway	5	5.1	3.8	3.68E-02
mmu04960:Aldosterone-regulated sodium reabsorption	3	3.0	9.4	3.83E-02
Tissue Specific expression				
Plasma	11	11.1	22.1	5.68E-11
Liver	46	46.5	2.5	7.09E-10
Skeletal muscle	7	7.1	7.5	3.31E-04
Heart	14	14.1	2.8	1.14E-03

Downregulated genes - Functional annotation enriched terms

Gene Ontology - Biological Process	**Count**	**%**	**Fold Enrichment**	**PValue**
GO:0019318~hexose metabolic process	4	6.5	9.2	8.45E-03
GO:0005996~monosaccharide metabolic process	4	6.5	8.2	1.17E-02
GO:0034613~cellular protein localization	4	6.5	5.1	4.03E-02
GO:0070727~cellular macromolecule localization	4	6.5	5.0	4.10E-02
GO:0006605~protein targeting	3	4.8	8.4	4.65E-02
GO:0006006~glucose metabolic process	3	4.8	8.4	4.72E-02
Gene Ontology - Cell Component				
GO:0070013~intracellular organelle lumen	9	14.5	2.2	4.67E-02
GO:0043233~organelle lumen	9	14.5	2.2	4.74E-02
Tissue Specific expression				
Macrophage	8	12.9	4.0	3.35E-03

table S4. **Microarray analyses on quadriceps muscle from Control f/f versus MERKO mice**. Significant differences in gene expression between genotypes are indicated within a 5% false discovery rate.

Gene expression reduced by muscle ERα deletion compared with Control f/f

PROBE_ID	SYMBOL	logFC	AveExpr	t (fold change f/fvMERK(	P.Value	adj.P.Val	B (log odds of differe
ILMN_2726412	**Esr1**	-2.277655474	6.473039909	-15.6036705	3.94E-08	0.000354148	8.444042165
ILMN_1234415	**Prima1**	-0.985307272	5.922511303	-8.6208823	8.07E-06	0.004137207	4.138486255
ILMN_2783833	**Gm1157**	-3.085046066	10.25763503	-8.195526888	1.24E-05	0.00573454	3.742885352
ILMN_2895511	**Kcng4**	-0.916773398	8.820014735	-7.474888938	2.69E-05	0.009552424	3.022823108
ILMN_2698004	**Gck**	-1.82891154	6.262887545	-7.388021686	2.96E-05	0.009954142	2.931550936
ILMN_2746870	**Nog**	-0.69628891	5.575008116	-7.126006382	3.99E-05	0.012596914	2.650141775
ILMN_2739544	**Stc2**	-1.342463315	7.027091096	-7.097193346	4.13E-05	0.012691305	2.618627264
ILMN_2712668	**C230090D14**	-0.509987365	11.73148254	-7.054088439	4.34E-05	0.012835171	2.571267566
ILMN_1230396	**Zc3hdc6**	-0.716443785	8.259988453	-7.020872651	4.51E-05	0.012835171	2.534597927
ILMN_2760468	**C230090D14**	-0.771836037	6.362214562	-6.596580615	7.45E-05	0.016923645	2.052505123
ILMN_1225229	**Slc35e4**	-0.774234383	6.757787034	-6.596411795	7.46E-05	0.016923645	2.052308167
ILMN_2679711	**Rabl4**	-0.667237744	9.322547575	-6.519299525	8.19E-05	0.01799905	1.961908427
ILMN_2803334	**Alg2**	-0.631338924	8.024634736	-6.499574137	8.39E-05	0.018150562	1.93864412
ILMN_2441534	**Tsga2**	-1.30528737	7.577639006	-6.368360398	9.86E-05	0.020082437	1.782426861
ILMN_2863362	**Mme**	-0.651761031	6.078654478	-6.318288424	0.000104977	0.021063814	1.722138623
ILMN_2658208	**Setd8**	-0.629431679	9.757696505	-6.277640071	0.00011045	0.021599321	1.67292066
ILMN_2603825	**Setd8**	-0.649898125	9.512540067	-6.127307171	0.000133536	0.023965408	1.488729066
ILMN_2698449	**Dtr**	-0.607784637	9.035870069	-6.040200797	0.000149264	0.026158932	1.380432058
ILMN_1248959	**Tinag**	-0.993880079	6.140244205	-5.980790646	0.000161133	0.027680702	1.305902065
ILMN_2881296	**Tmem66**	-1.071256243	11.00779167	-5.948800929	0.000167944	0.027680702	1.265545954
ILMN_2755443	**Sh3kbp1**	-0.537838853	9.99593112	-5.936257875	0.000170698	0.027803724	1.249679369
ILMN_2667091	**Ppp1r3c**	-0.901458163	14.03156243	-5.860796388	0.000188335	0.029630651	1.153709067
ILMN_3001076	**Setd8**	-0.525905614	10.33957584	-5.809672685	0.000201396	0.031329543	1.088188836
ILMN_1258578	**2310047C17Rik**	-0.454848465	11.14861774	-5.679750943	0.000239196	0.034859609	0.919846243
ILMN_1232195	**1110028E10Rik**	-0.412139153	8.524987459	-5.643614717	0.000251021	0.036201937	0.872553859
ILMN_1217723	**Stau2**	-0.488043202	9.579105896	-5.634241208	0.00025419	0.036281081	0.86025302
ILMN_2922560	**Slc16a9**	-0.588108227	7.261534577	-5.570052793	0.000277086	0.037786705	0.77564736
ILMN_1238640	**2310003H01Rik**	-0.590767006	9.060650926	-5.514334487	0.000298765	0.039022712	0.70167979
ILMN_3161419	**LOC627585**	-0.480763801	5.285061196	-5.466431405	0.000318866	0.039772059	0.637695467
ILMN_2613908	**Alox12**	-0.622291271	5.913561455	-5.418274376	0.000340547	0.041358534	0.573006443
ILMN_2966162	**Tmem56**	-0.50897192	10.60424426	-5.411029545	0.000343944	0.041407823	0.563242772
ILMN_2881019	**Sh3kbp1**	-0.501277423	10.30154117	-5.325876559	0.000386714	0.04499208	0.44786174
ILMN_2874084	**Car9**	-0.68628721	5.721217354	-5.272718561	0.000416288	0.047608333	0.375251436
ILMN_1220617	**Hr**	-0.952222415	8.056302289	-5.239345824	0.000436089	0.049086551	0.329437863

Gene expression elevated by muscle ERα deletion compared with Control f/f

ILMN_2852925	**Higd1b**	0.598853741	6.741874467	5.230483931	0.000441515	0.049296548	0.317242766
ILMN_2780760	**Pknox1**	0.408085019	6.961196673	5.267160404	0.000419517	0.047608333	0.367633523
ILMN_2838372	**2010111I01Rik**	0.560150022	7.976222684	5.313921987	0.000393162	0.045361117	0.431571586
ILMN_2426965	**Tpm3**	0.865956302	8.671598109	5.340772854	0.000378838	0.044449215	0.468128834
ILMN_2604224	**Sema5a**	0.418780647	6.504126226	5.351395281	0.000373326	0.044176952	0.482559673
ILMN_3009225	**Apoc4**	1.741132414	5.806519409	5.364176726	0.000366809	0.043779898	0.499899917
ILMN_3074610	**C730004C24Rik**	0.98603833	5.135629557	5.421293098	0.000339142	0.041358534	0.577072245
ILMN_2973089	**Coq3**	0.450959924	7.474659009	5.450362445	0.000325931	0.040290262	0.616150896
ILMN_2697749	**Glyat**	0.85041007	6.382990606	5.469734755	0.000317434	0.039772059	0.642119399
ILMN_1242984	**LOC245683**	0.598324881	7.452203619	5.481843266	0.000312245	0.039660802	0.658320692
ILMN_1214634	**Aqp9**	0.840557164	5.139415259	5.483947966	0.000311352	0.039660802	0.661134436
ILMN_2592266	**2310009N05Rik**	0.497823605	10.14074002	5.493980818	0.000307134	0.039660802	0.674537597
ILMN_2937735	**Irak2**	0.561623782	7.834088595	5.515557299	0.000298271	0.039022712	0.703308362
ILMN_2710274	**Slc9a3r2**	0.449430221	10.17514769	5.531951054	0.00029172	0.038835227	0.725119193
ILMN_2832105	**Fgg**	1.586463246	6.648757379	5.535908216	0.000290162	0.038835227	0.730377588
ILMN_2654700	**Drp2**	0.649022105	6.284021226	5.566583675	0.000278385	0.037786705	0.7710563
ILMN_2797973	**Pgk1**	0.487710438	5.767358592	5.57168359	0.000276478	0.037786705	0.777804917
ILMN_2660837	**Siat7d**	0.624994585	7.048523076	5.589017564	0.000270099	0.037772886	0.800711995
ILMN_2635871	**3110057O12Rik**	0.704887777	7.176053729	5.592814968	0.000268722	0.037772886	0.805723996
ILMN_1243507	**Ssb4**	0.803992506	5.794849565	5.71908927	0.000227001	0.033434385	0.971096477
ILMN_1225835	**Mfap5**	0.564727121	9.04575175	5.725564246	0.000225059	0.033434385	0.979508888
ILMN_2806065	**Ankrd2**	0.958794958	11.54685999	5.763891499	0.000213924	0.032193238	1.029170093
ILMN_2691059	**Cyp2c37**	1.413709263	5.727364734	5.785113088	0.000208015	0.031648044	1.056568519
ILMN_1247071	**Slc22a1**	0.683502696	5.188619269	5.785720724	0.000207849	0.031648044	1.05735198
ILMN_2907540	**Lmcd1**	0.529719773	12.74069909	5.865883845	0.000187086	0.029630651	1.160206937
ILMN_2655555	**Taf9**	0.544171166	5.095932542	5.876458617	0.000184519	0.029630651	1.173700532
ILMN_3127335	**Sytl2**	0.937081321	8.288181434	5.950815547	0.000167506	0.027680702	1.268092121
ILMN_1225699	**Serpina1a**	0.937753743	5.146544911	5.953045476	0.000167022	0.027680702	1.270909677
ILMN_1219231	**Akap1**	0.485741131	9.298170107	5.960431248	0.000165432	0.027680702	1.280236254
ILMN_2732401	**C130068O12Rik**	0.449211146	13.30461459	6.118648723	0.000135016	0.023965408	1.478016108
ILMN_2696171	**D830014E11Rik**	0.848716145	6.348573611	6.121222626	0.000134575	0.023965408	1.48120195
ILMN_2728473	**Serpina3m**	0.683309941	5.519770414	6.141890146	0.000131083	0.023965408	1.50674654
ILMN_2770386	**Fhl2**	0.638539351	5.249988058	6.20361382	0.000121226	0.022680766	1.582649266
ILMN_2828768	**Usp28**	0.468918176	9.518707654	6.218821891	0.000118923	0.022554584	1.601262268
ILMN_1225873	**Slc1a1**	1.001269515	7.719435452	6.240886222	0.000115664	0.022241314	1.628204448
ILMN_1228469	**Apoc1**	2.045885967	7.560372253	6.275358535	0.000110766	0.021599321	1.670150771

ILMN_2955818	**Ugt2b36**	1.853024782	5.923882464	6.383368051	9.68E-05	0.020006308	1.800423674
ILMN_2734924	**1190003J15Rik**	1.101645067	5.981411405	6.448128988	8.94E-05	0.018748148	1.877699569
ILMN_2789650	**Csrp3**	1.183661329	11.28958598	6.466806573	8.73E-05	0.018604273	1.899871145
ILMN_2753029	**Gch1**	0.650253979	6.127088124	6.567356461	7.72E-05	0.017248636	2.018348291
ILMN_2774563	**Mug1**	1.368534844	5.33041277	6.683493626	6.71E-05	0.015751412	2.153354321
ILMN_2613038	**1110033I14Rik**	1.085508191	12.45057323	6.797541924	5.86E-05	0.013981816	2.284037762
ILMN_1259180	**Dscr1**	1.094526735	8.3697549	6.847529884	5.52E-05	0.0134089	2.340731386
ILMN_1245514	**Cyp2c70**	1.654420375	5.604496221	6.851668477	5.49E-05	0.0134089	2.345409256
ILMN_2657822	**Stat2**	0.730806756	5.240634131	6.858335948	5.45E-05	0.0134089	2.352940427
ILMN_1247811	**Ass1**	0.781026835	7.251030268	6.877631597	5.33E-05	0.0134089	2.374700197
ILMN_1255164	**Slco1b2**	2.408492256	6.418395089	6.880954456	5.31E-05	0.0134089	2.378442086
ILMN_2742068	**Csrp3**	1.160151851	11.36891643	6.889526975	5.25E-05	0.0134089	2.388088453
ILMN_2589871	**Cd28**	0.857021794	5.750423038	6.913433089	5.11E-05	0.0134089	2.414934495
ILMN_1247343	**Lmcd1**	0.616618276	11.93803288	6.919977791	5.07E-05	0.0134089	2.422270031
ILMN_1234072	**Pdlim1**	0.782707772	11.50957436	7.013875362	4.54E-05	0.012835171	2.526853532
ILMN_2752524	**Paqr9**	0.881075506	7.040472056	7.040343643	4.41E-05	0.012835171	2.556112093
ILMN_2704749	**Es1**	1.054352193	5.812338994	7.123202959	4.00E-05	0.012596914	2.64708051
ILMN_1231765	**Cmya1**	0.843627567	10.16868429	7.371552326	3.02E-05	0.009954142	2.914133914
ILMN_2916705	**Tuba1a**	0.613987952	8.810120645	7.428113355	2.83E-05	0.009811187	2.973799288
ILMN_2847061	**Slc27a5**	0.812643276	5.039893743	7.51408854	2.58E-05	0.009387115	3.063685731
ILMN_2830333	**Hpxn**	1.105954661	5.558612461	7.529350041	2.53E-05	0.009387115	3.079540408
ILMN_1213817	**Mup3**	2.796324379	10.1177268	7.534169575	2.52E-05	0.009387115	3.084540961
ILMN_2952275	**Arg1**	2.123449516	5.977537015	7.566788302	2.43E-05	0.009387115	3.118305456
ILMN_2788223	**Kng1**	1.846166328	7.794218267	7.610715319	2.32E-05	0.009387115	3.163557694
ILMN_2599794	**Apoc1**	1.785353866	8.606037905	7.682349623	2.14E-05	0.008987802	3.236820465
ILMN_3159131	**Cyp2a5**	1.767174907	6.098027092	7.763288413	1.96E-05	0.008490419	3.318811525
ILMN_2993745	**Ahsg**	2.430013306	6.330040444	7.84604385	1.80E-05	0.008018286	3.40178775
ILMN_3133352	**Ung**	0.67859964	9.685683581	8.202872819	1.23E-05	0.00573454	3.749896549
ILMN_2840533	**Aldob**	1.492942539	5.58047853	8.244035893	1.18E-05	0.00573454	3.789065404
ILMN_2645815	**Cyp2a12**	0.883215487	5.201710906	8.709923684	7.39E-06	0.003933361	4.218689512
ILMN_2789651	**Csrp3**	1.290126546	9.416025633	8.836374125	6.52E-06	0.003613195	4.331082385
ILMN_1236304	**Hamp**	2.553088467	6.242150275	8.95166173	5.83E-06	0.003365188	4.432036711
ILMN_2602185	**40795**	0.765137244	5.810790625	8.999171227	5.57E-06	0.00335434	4.473224342
ILMN_2788221	**Kng1**	1.572570744	5.850510617	9.43980163	3.68E-06	0.002314376	4.844000915
ILMN_2980371	**Es1**	1.897095548	5.701402114	9.480549614	3.54E-06	0.002314376	4.877291163
ILMN_1247156	**Apoa2**	3.481417885	9.463187033	9.555139963	3.31E-06	0.002289433	4.937803731
ILMN_2837080	**Azgp1**	2.677402425	6.973847797	9.694849363	2.91E-06	0.002122158	5.049679882

ILMN_1235719	**LOC231396**	2.587061718	6.354844262	9.73824757	2.80E-06	0.002122158	5.084048039
ILMN_2718431	**Itih4**	1.49688907	5.725354034	9.954857314	2.31E-06	0.001879507	5.252915572
ILMN_2764036	**Ahsg**	2.531040376	6.093317727	9.994000668	2.23E-06	0.001879507	5.282963367
ILMN_2904137	**Ambp**	2.784319379	6.884209999	10.2238133	1.82E-06	0.001683418	5.45655142
ILMN_1232567	**Fga**	2.697542634	6.452350414	11.00730477	9.46E-07	0.00093527	6.01383795
ILMN_2644185	**Apof**	1.292275019	5.584615195	11.01243614	9.42E-07	0.00093527	6.017320168
ILMN_2678460	**Mat1a**	2.375178305	6.493208917	11.39133128	6.96E-07	0.000802485	6.268739707
ILMN_2443330	**Ttr**	2.964318941	8.471966781	11.55506845	6.12E-07	0.000770051	6.373994189
ILMN_1220234	**Serpina1e**	3.593539271	7.10418024	12.72452138	2.56E-07	0.000354148	7.070693479
ILMN_1225570	**Serpina1d**	3.922791543	7.824788714	12.84934757	2.34E-07	0.000354148	7.139724057
ILMN_2659680	**Serpina1b**	3.761521553	8.190775075	12.93341894	2.21E-07	0.000354148	7.185672618
ILMN_1215859	**Serpina1b**	4.190034574	7.774562696	13.03215526	2.06E-07	0.000354148	7.239084781
ILMN_3004600	**Serpina1c**	3.842734479	7.217742062	13.07243128	2.00E-07	0.000354148	7.260703081
ILMN_2744565	**Hpxn**	2.956554723	6.605820876	13.20790998	1.82E-07	0.000354148	7.332710518
ILMN_2624363	**Fga**	2.922938932	6.539895689	13.30342043	1.71E-07	0.000354148	7.38282369
ILMN_2623393	**Apoa1**	3.689650158	7.3051642	13.63416558	1.36E-07	0.000354148	7.552305039
ILMN_2651539	**Apoc3**	3.046626036	6.576112981	14.93852279	5.88E-08	0.000354148	8.163806332

table S5. Primer sequences for qPCR analyses on tissues and for genotyping of Control f/f and MERKO mice.

Q-PCR			
GeneSymbol	NCBI Ref.Seq	Forward	Reverse
Acaca	NM_133360	CTGAAGCAGATCCGCAGCTT	GGTGAGATGTGCTGGGTCATG
Acacb	NM_133904	CATACACAGAGCTGGTGTTGGACT	CACCATGCCCACCTCGTTAC
Acadm	NM_007382	GCAGCCAATGATGTGTGCTTAC	CACCCTTCTTCTCTGCTTTGGT
Acox1	NM_015729	GCCCAACTGTGACTTCCAT	GGCATGTAACCCGTAGCACT
Atg12	NM_026217	CCACAGCCCATTTCTTTGTT	GAAACAGCCACCCCAGAG
Atg4b	NM_174874	TCGGACAGGGAAGATGGAC	AAATGCTGTATTTTCTGCCCA
Atg5	NM_053069	ACAGCTTCTGGATGAAAGGC	TGGGACTGCAGAATGACAGA
Atp5A1	NM_007505	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
Cd36	NM_007643	TCCAGCCAATGCCTTTGC	TGGAGATTACTTTTTCAGTGCAGAA
Cox7a1	NM_009944	CTCTTCCAGGCCGACAATGA	CCCAGCCCAAGCAGTATAAGC
Cpt1b	NM_009948	CTCCTGGAAGAAACGCCTTATT	CACCTTGCAGTAGTTGGAACC
Cyc1	NM_025567	TGGCTCCTCCCATCTACACAG	CGATGGTCATGCTCTGGTTCT
Cycs	NM_007808	CCAAATCTCCACGGTCTGTTC	ATCAGGGTATCCTCTCCCCAG
Err1	NM_007953	GGAGGACGGCAGAAGTACAAA	GCGACACCAGAGCGTTCAC
Errg	NM_011935	TTGTACTTCTGCCGACCTCC	TGAGATCACAAAGCGCAGAC
Esr1(ex4-5)	NM_007956	GCTACTGTGCCGTGTGCAA	TGTCAATGGTGCATTGGTTTG
Esr1 (ex 3)	NM_007956	GCCAGAATGGCCGAGAGAG	CCCCATAATGGTAGCCAGAGG
Esr2	NM_010157	GCCAACCTCCTGATGCTTCT	TCGTACACCGGGACCACAT
Fasn	NM_007988	TGCTCCCAGCTGCAGGC	GCCCGGTAGCTCTGGGTGTA
Fbxo32	NM_026346	TCAGGGATGTGAGCTGTGAC	AAGGAGCGCCATGGATACT
Gata1	NM_008089.1	AGGGCAGAATCCACAAACTG	AGGGCAGAATCCACAAACTG
Gata3	NM_008091.3	GCCTGCGGACTCTACCATAA	CATTAGCGTTCCTCCTCCAG
Gper	NM_029771	CCAAGCCTCAACACTCACACA	CCTGGGAGCCTGTTAGTCTCA
Gpx3	NM_008161	GATGGTGAGGGCTCCATACT	CATCCTGCCTTCTGTCCCT
Lmcd1	NM_144799	ATGGCAAAAGTGGCTAAGGA	TTGCATATTTTCCTCCACGA
Map1lc3b	NM_026160	AATCACTGGGATCTTGGTGG	AGTCAGATCGTCTGGCTCG
Mef2c	NM_025282	GCCGGACAAACTCAGACATTG	GGGTTTCCCAGTGTGCTGAC
mt-Co3	NC_005089	GCAGGATTCTTCTGAGCGTTCT	GTCAGCAGCCTCCTAGATCATGT
Ndufs1	NM_145518	CACTCGTTCCACCTCAGCTA	GACGGCTCCTCTACTGCCT
Nrf1	NM_010938	GAACTGCCAACCACAGTCAC	CGTCTGGATGGTCATTTCAC
Park7	NM_020569	AACACACCCACTGGCTAAGG	GTGCCTCCACAATGGCTAGT
Pik3c3	NM_181414	GTTCAGCTTCATCGGAGGAG	ATTCTTGGAGTTGGAGACCG
Polg1	NM_017462	TAGCTGGCTGGTCCAAGAGT	CGACGTGGAGGTCTGCTT
Ppard	NM_011145	GCCTCGGGCTTCCACTAC	AGATCCGATCGCACTTCTCA
Pparg	NM_011146	GCCCTTTGGTGACTTTATGG	CAGCAGGTTGTCTTGGATGT
Ppargc1	NM_008904	TGAGGACCGCTAGCAAGTTT	TGAAGTGGTGTAGCGACCAA
Ppargc1b	NM_133249	CTGAGTCAAAGTCACTGGCG	GCTCTCGTCCTTCTTCCTCA
Ppia	NM_008907	AGCCAAATCCTTTCTCTCCAG	CACCGTGTTCTTCGACATCA
Sdha	NM_023281	TACTACAGCCCCAAGTCT	TGGACCCATCTTCTATGC
SLC2a4	NM_009204	CCCCCGATACCTCTACATCATC	GCATCAGACACATCAGCCCAG
Sqstm1	NM_011018	TTTCTGGGGTAGTGGGTGTC	CTGAAGAATGTGGGGGAGAG
Tfam	NM_009360	AGCTTGTAAATGAGGCTTGGA	AGATGTCTCCGGATCGTTTC
Ulk1	NM_009469	TAGTCAGCCAGGTCTCCACC	CTGCTGGGAAAGGAAATCAA
Ulk2	NM_013881	TAATCTGCCAGGTCTCCACC	CAAATTCTGCTTGGAAAGGAA
Mitochondrial Mutations			
Mt-copy		CCTATCACCCTTGCCATCAT	GAGGCTGTTGCTTGTGTGAC
Mt-flanking TaqI 634 site		ACTCAAAGGACTTGGCGGTA	AGCCCATTTCTTCCCATTTC

Genotyping		
Floxed ERα Exon 3	GACTCGCTACTGTGCCGTGTGC	CTTCCCTGGCATTACCACTTCTCCT
CRE transgene	GCATTACCGGTCGTAGCAACGAGTG	GAACGCTAGAGCCTGTTTTGCACGTTC

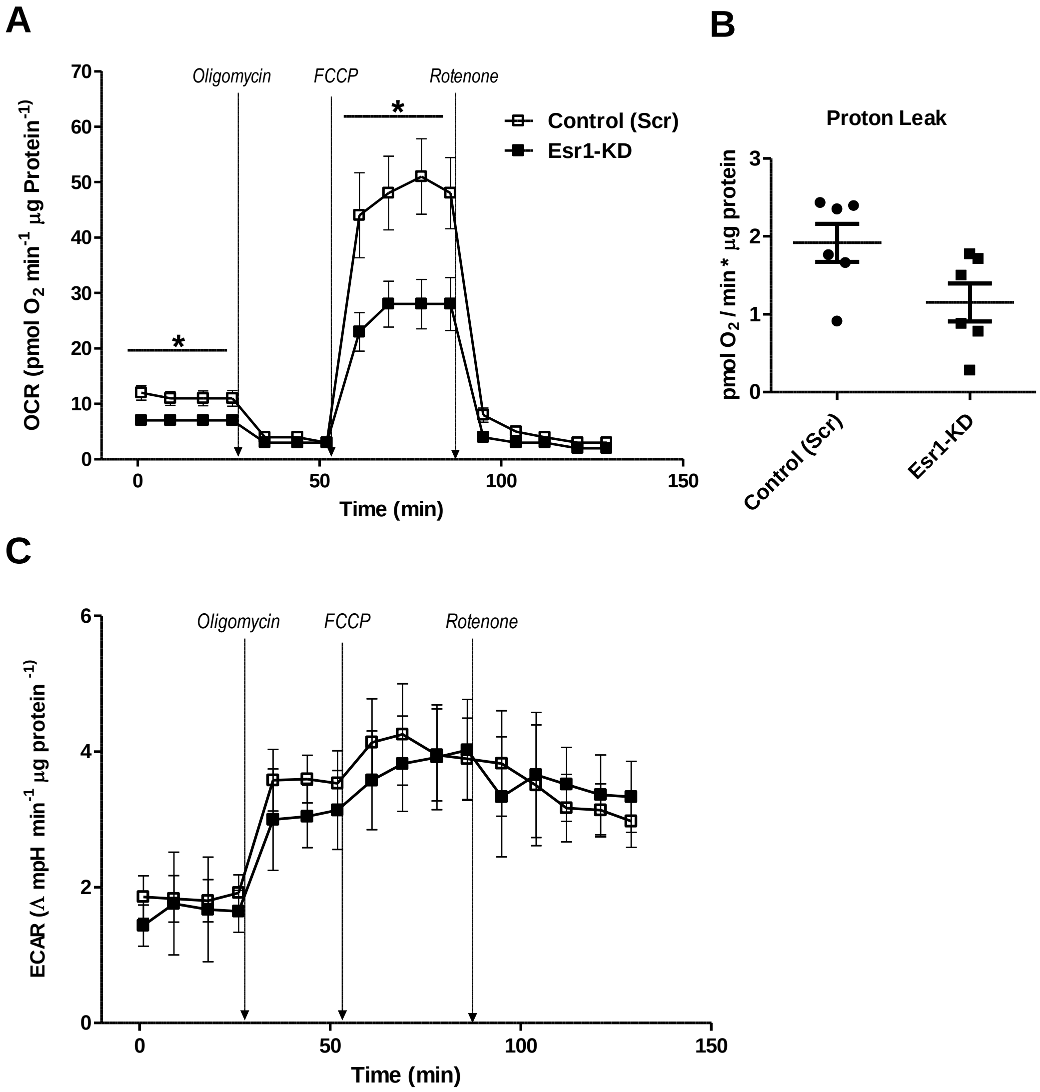

fig. S1. Real-time respirometry in C2C12 myotubes. (A) Traces of cellular oxygen consumption (OCR), (B) proton leak, and (C) extracellular acidification rate (ECAR) in Control (Scr) and Esr1-KD myotubes (*n*=6/genotype). Values are expressed as mean ± SEM and differences between genotypes were detected by Student's t-test and repeated measures ANOVA where appropriate, * *P*<0.05.

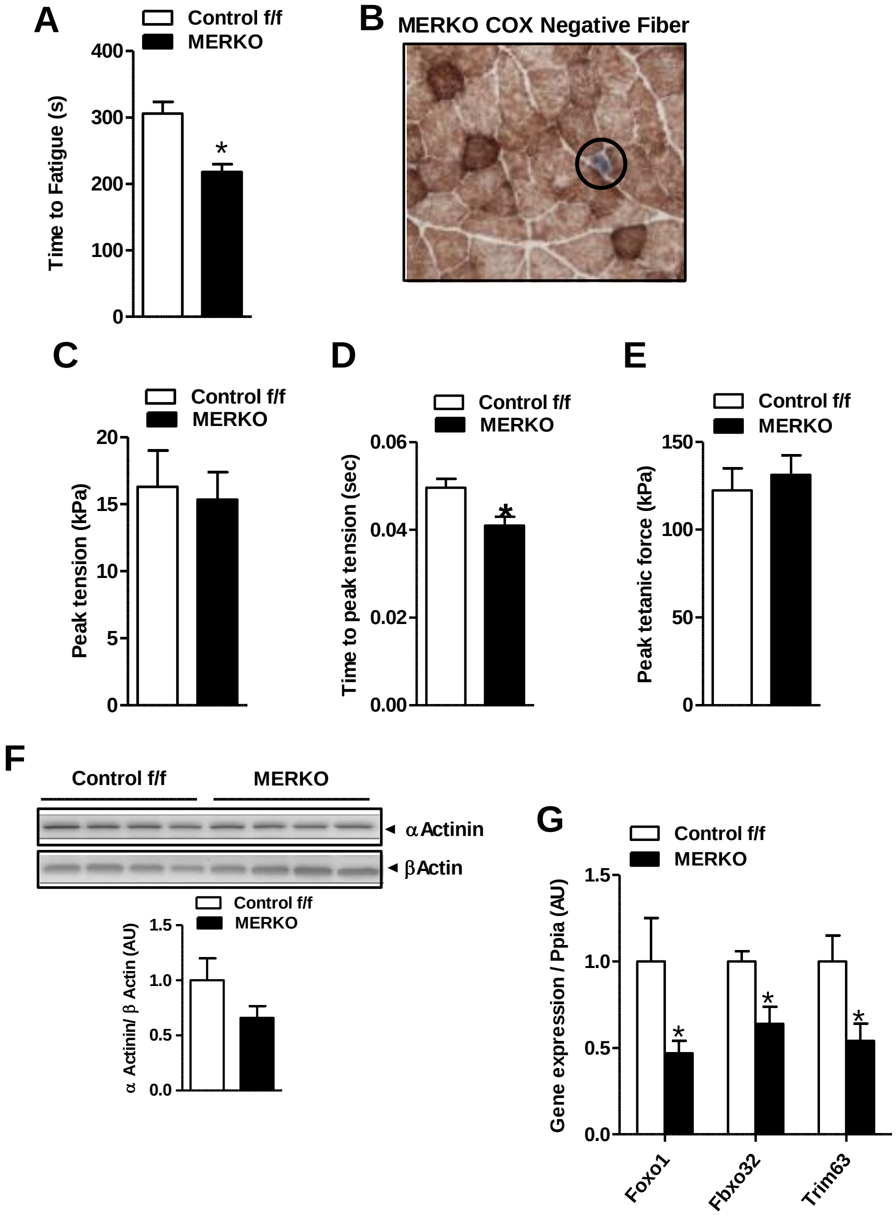

fig. S2. **Muscle peak tension and time to fatigue**. Solues muscle fiber (**A**) time to fatigue (**B**) cytochrome oxidase (COX) negative fibers identified only in MERKO muscle (n=5 positive of 6 MERKO mice observed), (**C**) peak tension, (**D**) time to peak tension, (**E**) peak tetanic force, (**F**) α Actinin protein abundance detected by immunoblotting, and (**G**) atrogene expression assessed by qPCR. All studies performed in muscle from female Control f/f (open bars) versus MERKO (closed bars) n=6 per genotype. Values are expressed as mean ± SEM and differences between groups were detected by Student's t-test, * $p<0.05$.

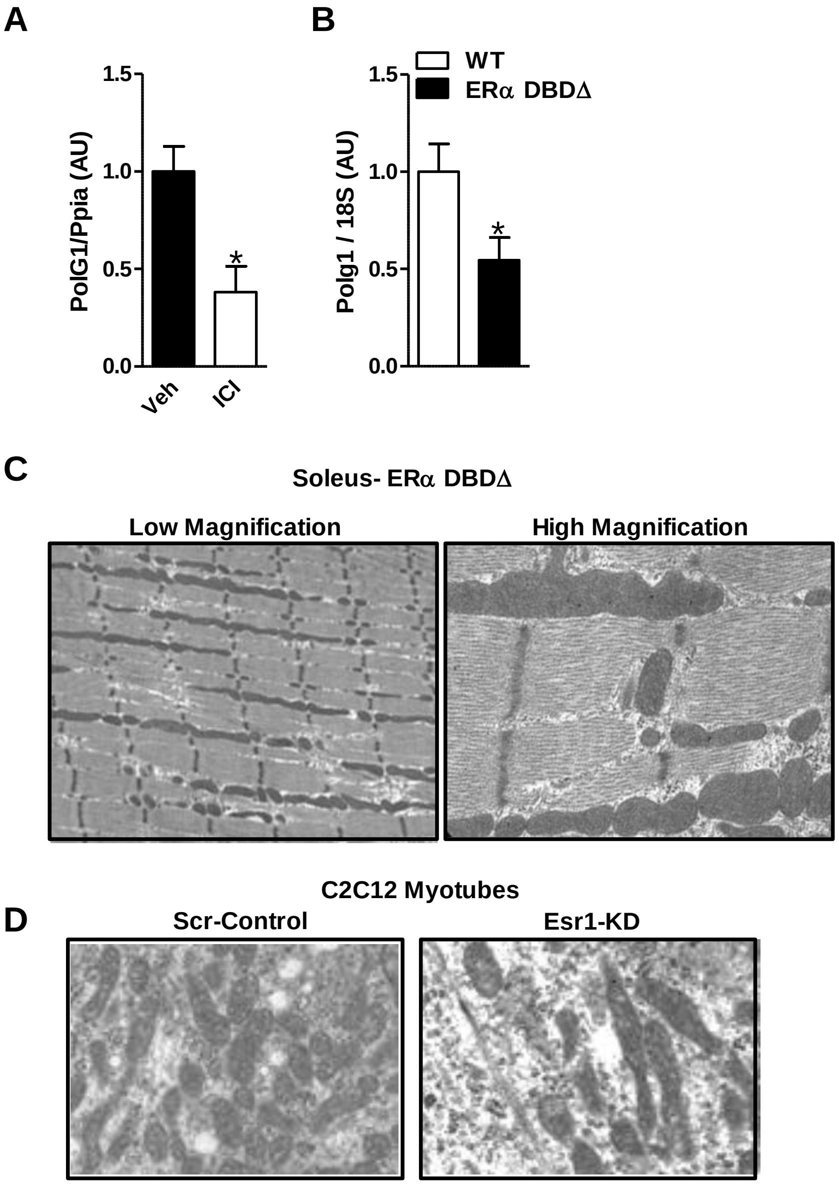

fig. S3. Polg1 expression is regulated by ERα in muscle. Polg1 expression levels were assessed in (**A**) C2C12 myotubes treated with ICI (1μM) for 24h and in (**B**) quadriceps muscle from ERα DBDΔ mice by qRT-PCR (normalized to Ppia). (**C**) Representative low and high magnification electron micrographs of soleus muscle from ERα DBDΔ mice. (**D**) Transmission electron microscopy of C2C12 myocytes shows elongated and fused mitochondria in Esr1-KD versus Control (Scr), morphology changes similar to that observed in MERKO versus Control f/f mouse muscle. Values are expressed as mean ± SEM and differences between groups were detected by one-way ANOVA , * $P<0.05$ compared to vehicle control.

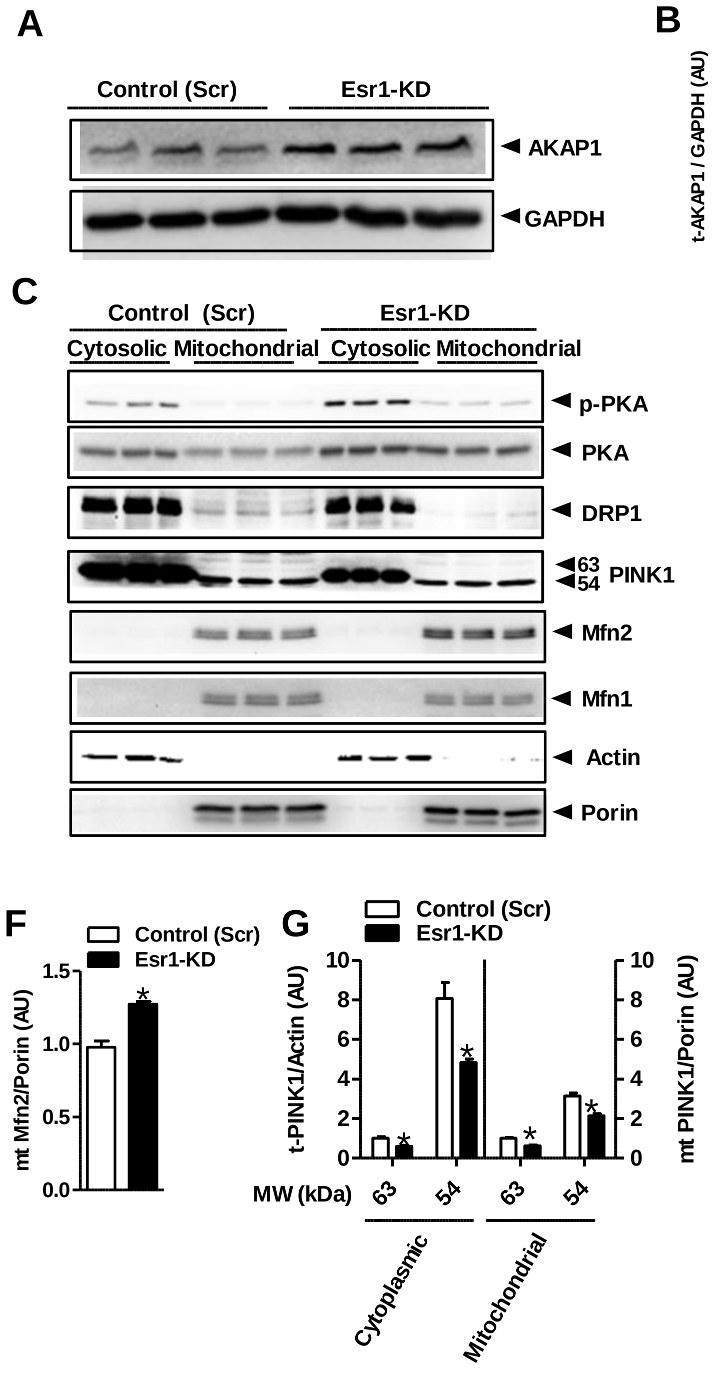

fig. S4. Altered mitochondrial fission-fusion signaling in ERα-deficient C2C12 mytoubes. (**A**) AKAP 1 protein abundance in Control (Scr) (open bars) and Esr1-KD (closed bars) myotubes as detected by immunoblotting and (**B**) densitometric analysis, n=6 / condition. (**C**) Protein abundance of (**D**) PKA, (**E**) DRP1, (**F**) Mfn1/2, and (**G**) PINK1 in Control (Scr) and Esr1-KD myotubes as detected by immunoblotting and densitometric analysis, n=6 / condition. Values are expressed as mean ± SEM and differences between groups were detected by Student's t-test and one-way ANOVA where appropriate, * *P*<0.05.

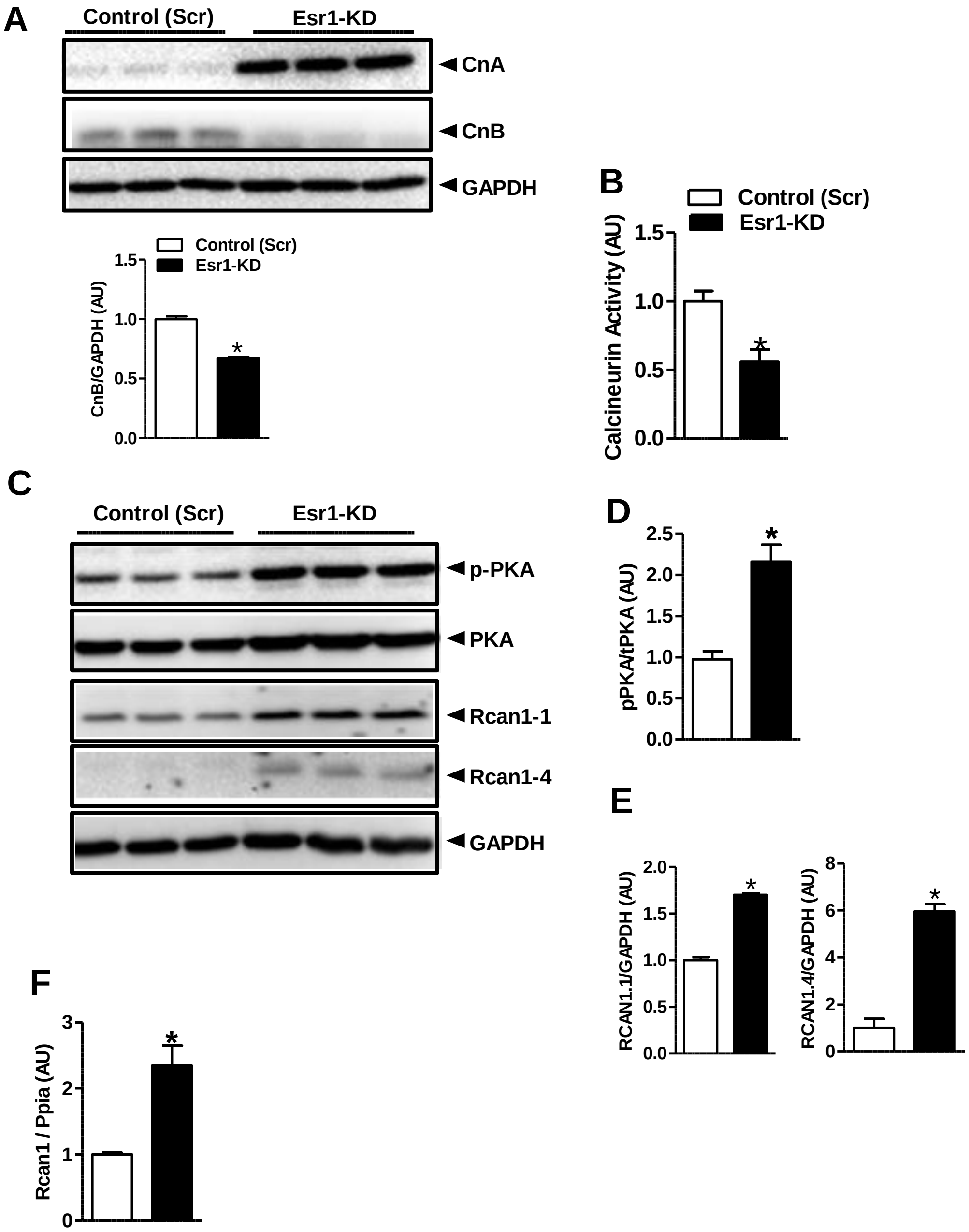

fig. S5. Calcineurin-PKA-Rcan1 axis in C2C12 myotubes with Esr1-KD. (**A**) Calcineurin subunit protein level and (**B**) enzymatic activity in Control (Scr) and Esr1-KD C2C12 myotubes. (**C-E**) Representative immunoblots (n=3) and corresponding densitometry (n=6) of total and phosphorylated PKA and Rcan1 (1-1, 38 kDa and 1-4, 26 kDa) normalized to GAPDH. (**F**) Rcan1 expression levels in Control (Scr) and Esr1-KD C2C12 myotubes normalized to Ppia (n=6/genotype). Values are expressed as mean ± SEM, and differences between groups were detected by Student's t-test, * *P*<0.05.

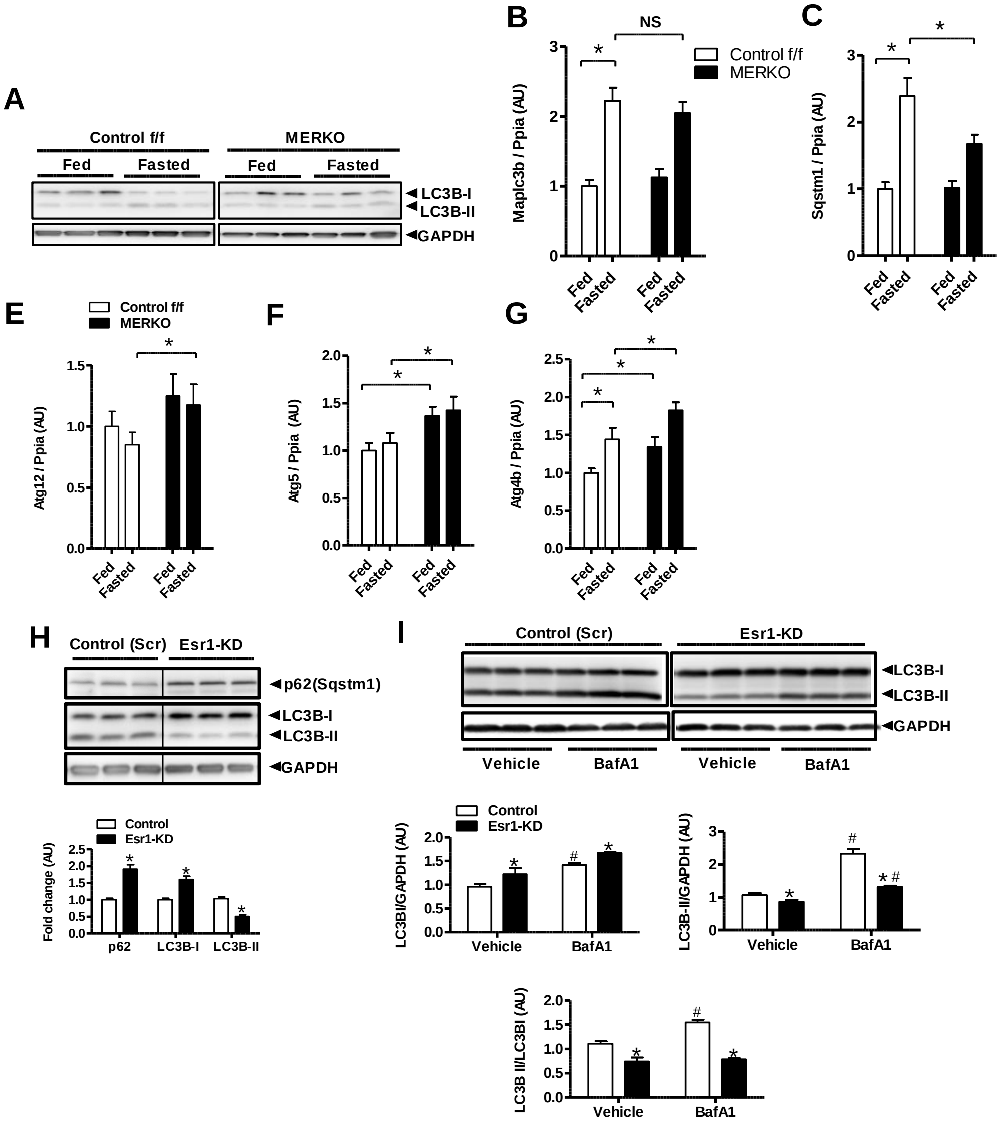

fig. S6. Expression of autophagy-related genes in MERKO muscle. (**A**) Representative immunoblots of LC3BI and II protein in muscle from fed and fasted (24h) Control f/f vs. MERKO mice (*n*=6 mice per condition). (**B-G**) Quantitative RT-PCR analyses of critical autophagic effectors in muscle from fed and fasted Control f/f and MERKO mice, *n*=6 mice/condition. (**B**) Maplc3b Atg7, (**C**) Sqstm1/p62, (**D**) Atg7, (**E**) Atg12, (**F**) Atg5, and (**G**) Atg4b muscle expression levels at basal and following fasting (24-h) in female Control f/f vs. MERKO mice (n=6 mice/condition). (**H-I**) p62 and LC3BI and II protein levels in Control (Scr) and Esr1-KD myotubes at basal and following bafilomycin A$_1$ (BafA$_1$, 25nM) treatment (*n*=3-6/condition). Values are expressed as mean ± SEM and differences between groups were detected by Student's *t* test one-way ANOVA. *$P<0.05$ difference between genotypes. $^{\#}$ $P<0.05$ within group, between treatment difference.

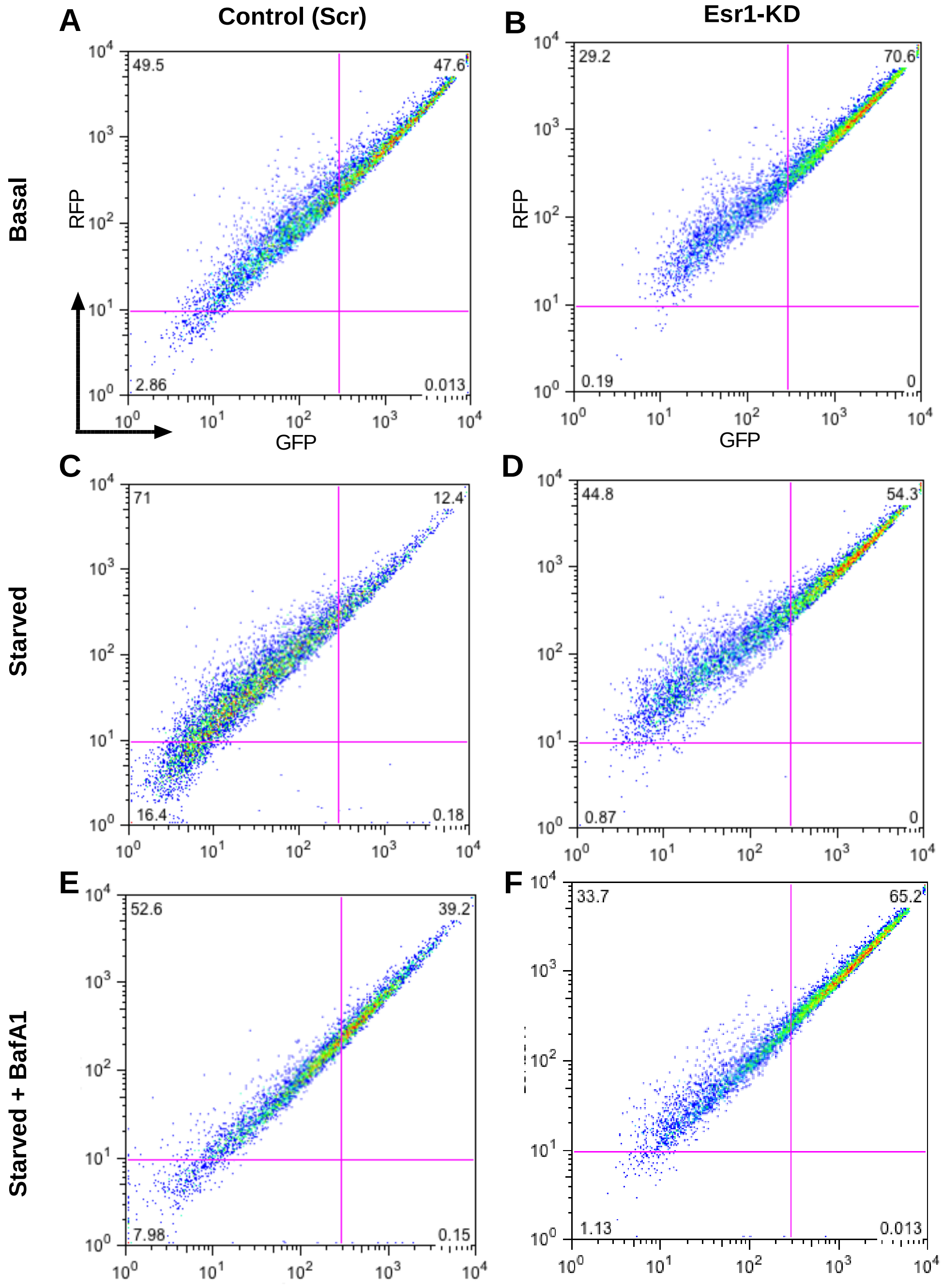

fig. S7. Autophagic flux studies in C2C12 myocytes with Esr1-KD. Flow cytometry analyses were performed on myocytes infected with tandem labeled Gfp-Rfp-Lc3b and exposed to nutrient deprivation (starvation) in the presence or absence of BafA$_1$. Representative FACS plots depicting autophagic flux in Scr-Control and Esr1-KD C2C12 myocytes under basal conditions (**A-B**), and following 4-hour nutrient deprivation (starvation) without (**C-D**) or with 4-hour BafA$_1$ treatment (**E-F**). Gates defining GFP-RFP double-positive and RFP single-positive cells show reduced autophagosome turnover (loss of GFP-RFP signal) and autolysosome formation (RFP single-positive signal) at basal and following starvation in Esr1-KD myocytes. Values presented in the far corners of quadrants indicate percent cells in each quadrant. Studies were performed in triplicate.