Supplemental Legends.

Fig. 1 Gonadotroph levels.

Blood was collected between 9 to10am at different stages of the estrous cycle. A. LH levels were not significantly different between groups. B. Control FSH levels were significantly higher compared to PitARKO mice in all stages of cyclicity. N=6-15.

Fig. 2 Quantitative real-time PCR.

A. *Nr5A1* (SF-1) expression in KO mice was significantly decreased when compared to control after OVX, however, there was no difference between control and KO at basal and after estradiol replacement B. and C. *BMP2* and *Nr5A2* were also measured and there was similar expression between control and KO at basal, after OVX and estradiol replacement. N=6-11.

Fig. 3 *Gnrh* mRNA expression in AVPV of hypothalamus.

Rostral hypothalamus, including the AVPV, was isolated and *Gnrh* mRNA was measured from control and PitARKO mice. There was no significant difference between control and PitARKO mice, intact, OVX and OVX+E2 stage. N=6-12

Fig. 4 AR knockout allele in pituitary genomic DNA.

Tissues were collected from ARfl/fl; αCre+ mouse. Genomic DNA isolation and PCR were performed as described in materials and methods. AR floxed allele was observeed in tail, OV (ovary), pit (pituitary), hyp (hypothalamus) and liv (liver). However, AR knockout allele was only observed in pituitary, and not in tail, ovary, hypothalamus and liver.

Fig. 5 mRNA expression, ovary follicles and fertility.

A. Thyroid stimulating hormone beta subunit (*TSHβ*) was measured from pituitary. B-D Steroidogenic enzyme mRNA expressions: *StAR*, *Cyp17* and *Cyp19* mRNA were measured from ovary. There was no significant difference among ARfl/fl;acre-; ARfl/wt;acre- and PitARKO mice. E. Corpora Lutea (CL), antral follicles and preantral follicles (primodial, primary, secondary follicles) were examined and only KO mice showed a significant reduction in CL. N=6-9. F-H. Female mice were introduced with a proven fertile male WT mouse and the total numbers of litters per female were recorded during the 90 days. There was no significant difference among ARfl/wt;acre-, ARfl/fl; acre- and KO groups (F). Number of pups per mouse per litter was significantly reduced in KO mice compared to ARfl/wt;acre-, ARfl/fl; acre- mice during the 90 days (G). There was no significant difference between ARwt/wt;acre- and ARwt/wt;acre+ groups (H). N=6.

Fig. 6 Western blot of AR.

Western blot was performed as in Fig. 1 B1 with protein molecular marker loaded in the first well. We observed reduced protein levels of AR in the pituitary but not in other tissues collected.

Fig. 7 Immunostaining of AR and LH showing co-localization in the mouse pituitary of control mouse. LH (Green fluorescence) expressed in the cytoplasm; AR (Red fluorescence) expressed mainly in nucleus. A. Sections under 10x objective fluorescence microscope. B. Sections under 40x objective fluorescence microscope. Arrows point to the nucleus in which AR and LH colocalize in the merged panel of control pituitary.

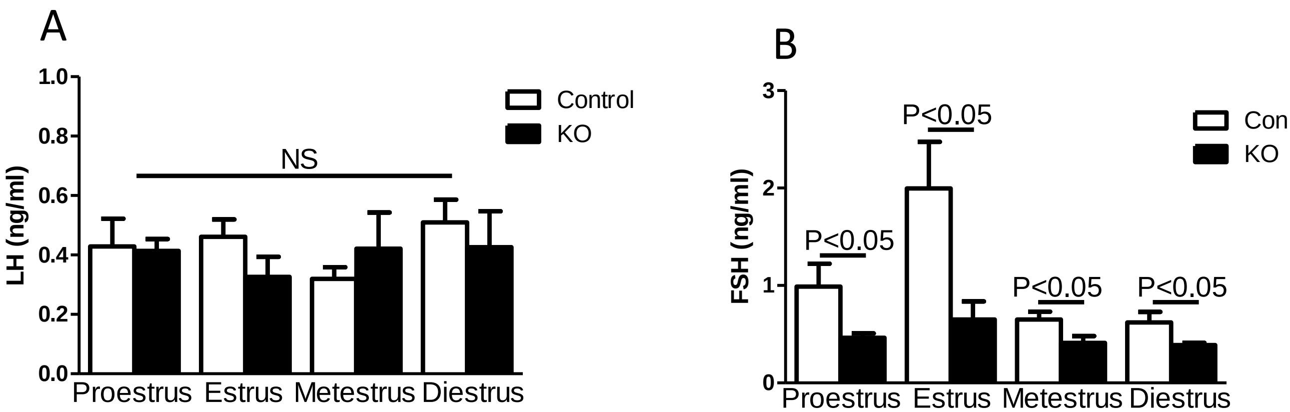
Sections of pituitaries (6 pituitaries/group) were evaluated under the 40x objective fluorescence microscope by counting all of the immunodetectable LH expressing cells and those LH expressing cells that also express nucleated AR from random selected area of each anterior pituitary. 99% of gonadotropes (gonadotropic cells) express nucleated AR co-localization within LH cytoplasm in control mice and 98% of gonadotropes do not express nucleated AR in PitARKO mice.

Supplemental data

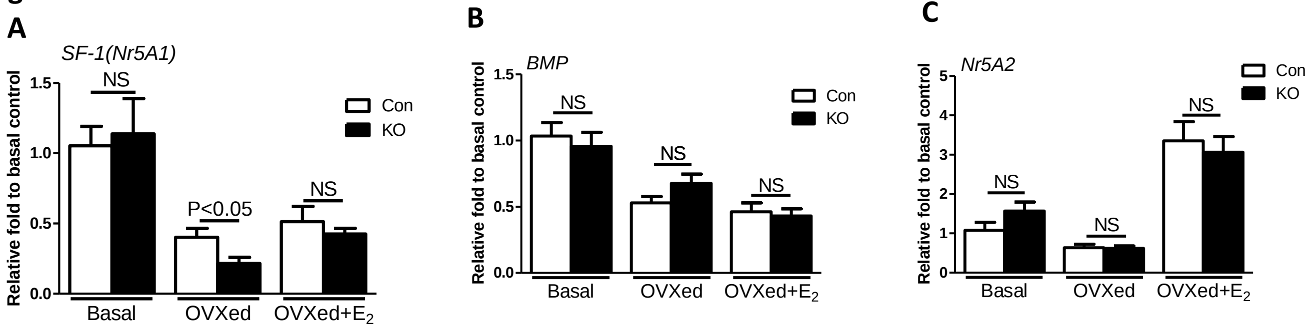
Table 1. Primers

F-forward; R-reverse		Primers	References
lhβ	F	GGCCGCAGAGAATGAGTTCTG	
	R	CTGAGGCACAGGAGGCAAA	
	Probe	CATGGTCCGAGTACTGCCGGCTG	
fshβ	F	GCCGTTTCTGCATAAGC	
	R	CAATCTTACGGTCTCGTATACC	
	Probe	CAATACCACTTGGTGTGCGGGCTA	
gnrh	F	CCAACGGAAGCTCGAGATCC	
	R	TGCCGGCCATCAGTTTGAG	
	Probe	TGACTTTCACATCCAAACAGAGTGGACA	
AR	F	GGCGGTCCTTCACTAATGTCAACT	
	R	GAGACTTGTGCATGCGGTACTCAT	
	Probe	CCAGGATGCTCTACTTTGCACCTGA	
activinA	F	ATCATCACCTTTGCCGAGTC	1
	R	CCCTTTAAGCCCATTTCCTC	1
activinb	F	ATCATCACCTTTGCCGAGTC	1
	R	CCCTTTAAGCCCATTTCCTC	1
prolactin	F	CAAGGAACAAGCCCTGAAAG	1
	R	ATCCCATTTCCTTTGGCTTC	1
pacap1	F	CCTATCGAAAAGTCTTGGAC	adapted from 2
	R	TTGACAGCCATTTGTTTTCG	adapted from 2
Nr5a1	F	ATC GAC AAG ACG CAG CGT AA	3
	R	CCC ACC GTC AGG CAC TTC	3
Nr5a2	F	CCA GAC CCT GTT CTC CAT TGT T	3
	R	CAT TTG GTC ATC AAC CTT CAG TTC	3
BMP2	F	GCTCCACAAACGAGAAAAGC	1
	R	AGCAAGGGGAAAAGGACACT	1
StAR	F	CCCAAAGAAGGCATAGCAAG	
	R	GCTGAATCCCCCAAACTTCT	
CYP17	F	GATCTAAGAAGCGCTCAGGCA	
	R	GGGCACTGCATCACGATAAA	
CYP19	F	TTGGAAATGCTGAACCCCAT	
	R	CAAGAATCTGCCATGGGAAA	
gnrhR	F	AAGACCCACGCAAACTACAGC	
	R	GTCCAGCAGACGACAAAGGAG	
	Probe	TCCAAGAATAATATCCCAAGAGCTCGGC	
gapdh	F	GGGCATCTTGGGCTACACT	
	R	GGCATCGAAGGTGGAAGAGT	
	Probe	AGGACCAGGTTGTCTCCTGCGA	
TSHβ	F	TCAACACCACCATCTGTGCT	1
	R	TCTGACAGCCTCGTGTATGC	1

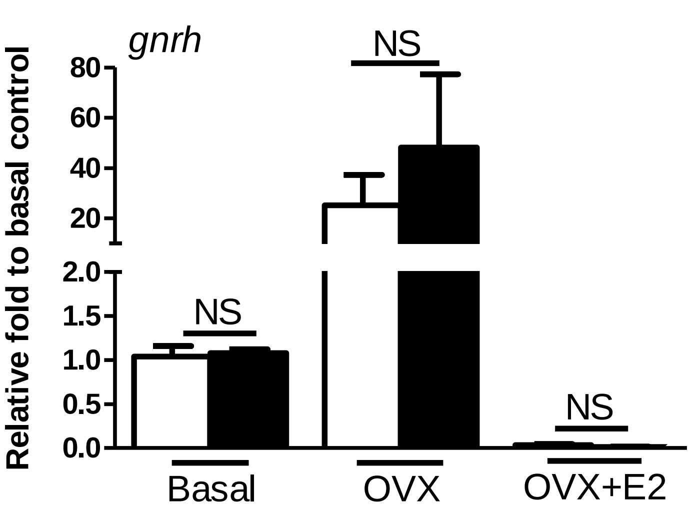
Suppl. Fig. 1



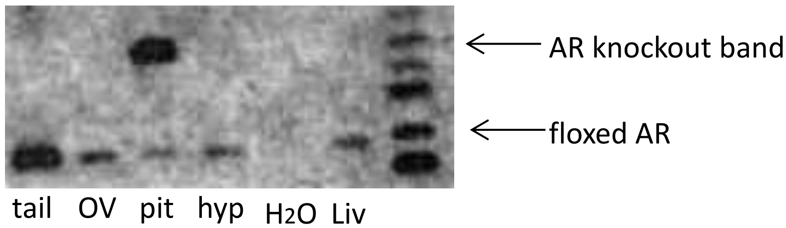
Suppl. Fig. 2



Suppl. Fig. 3



Suppl. Fig. 4



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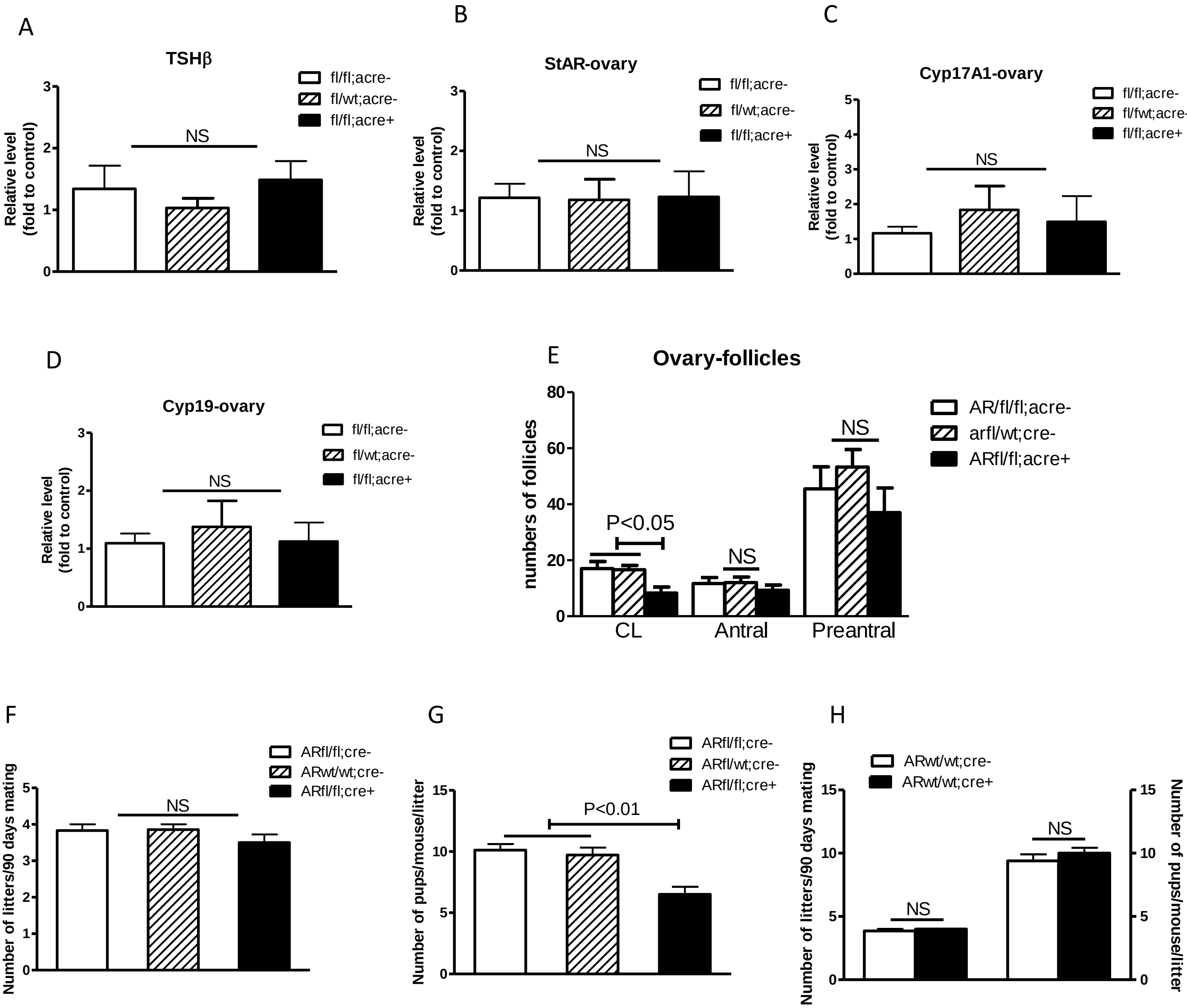

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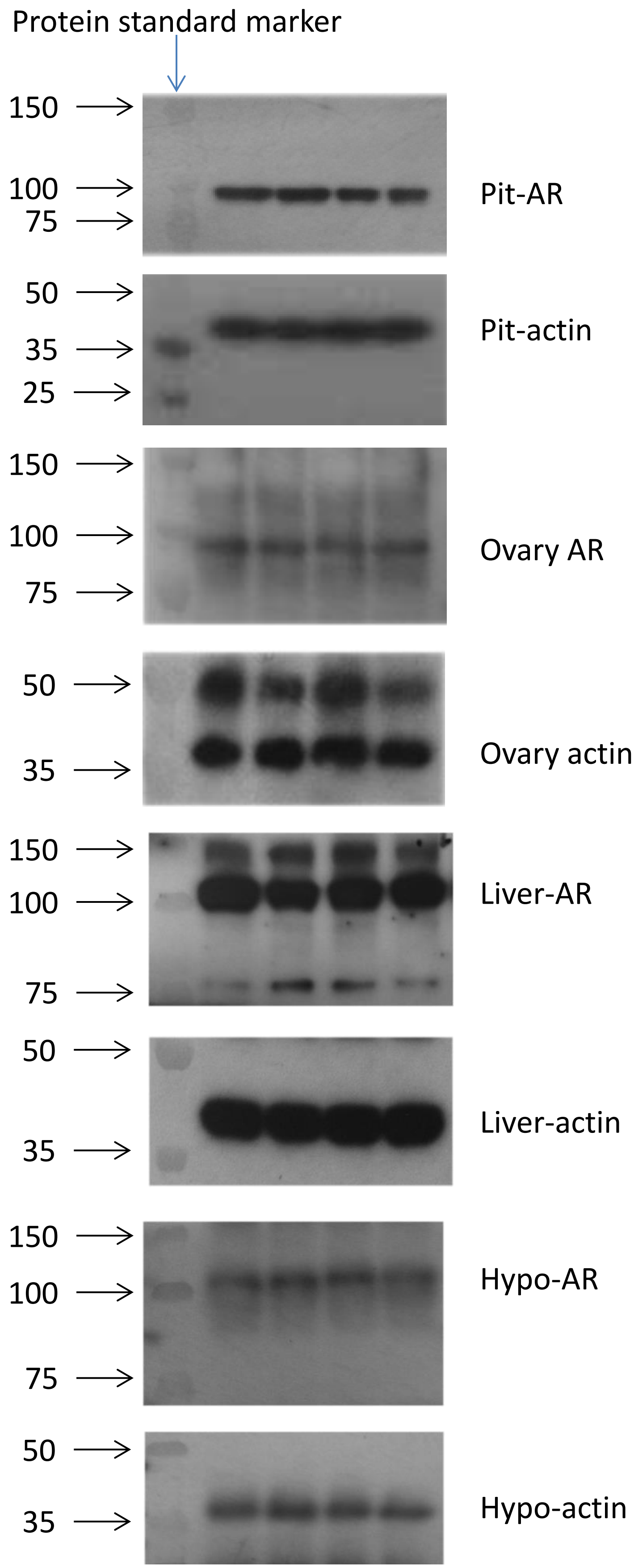
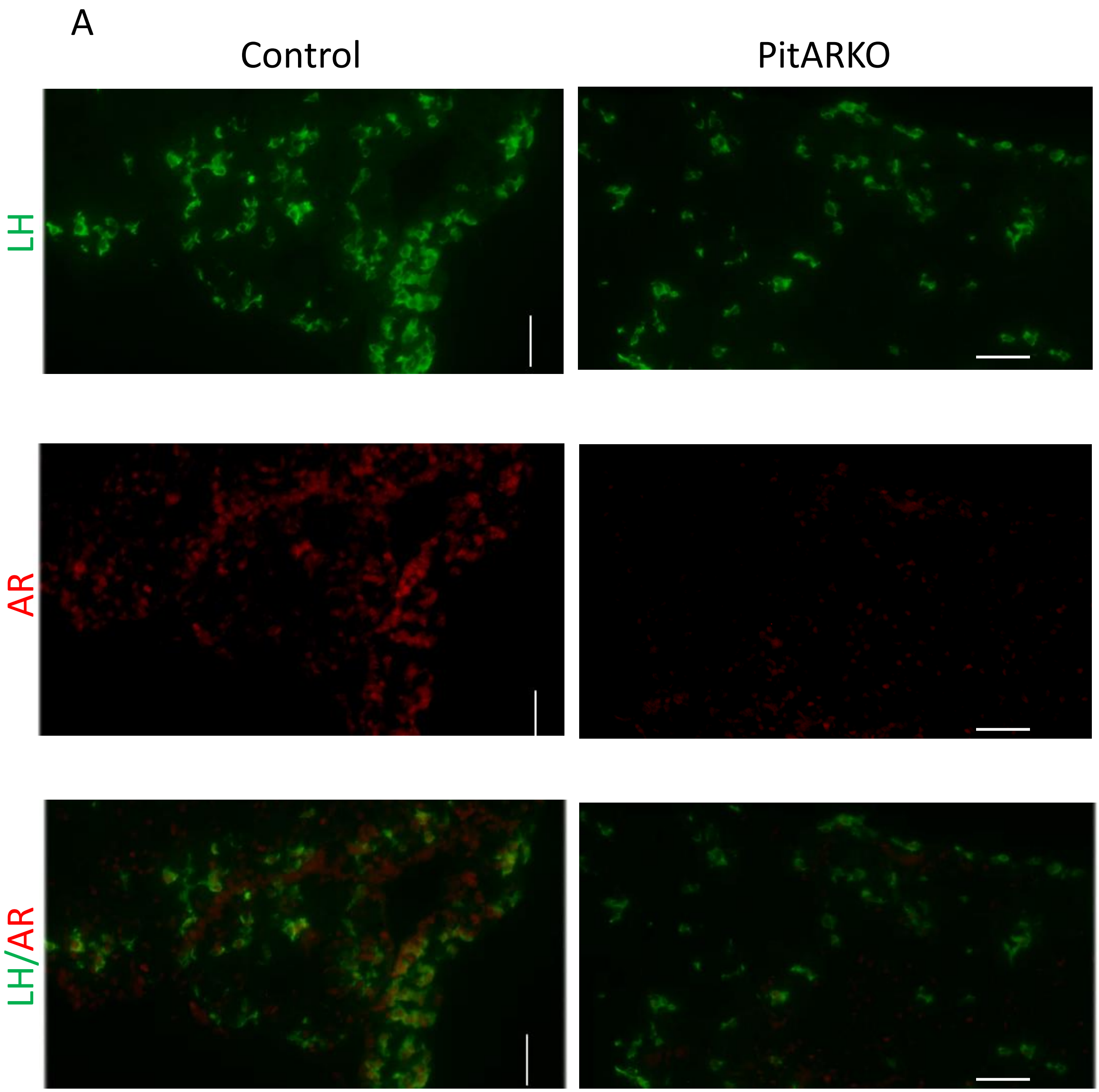
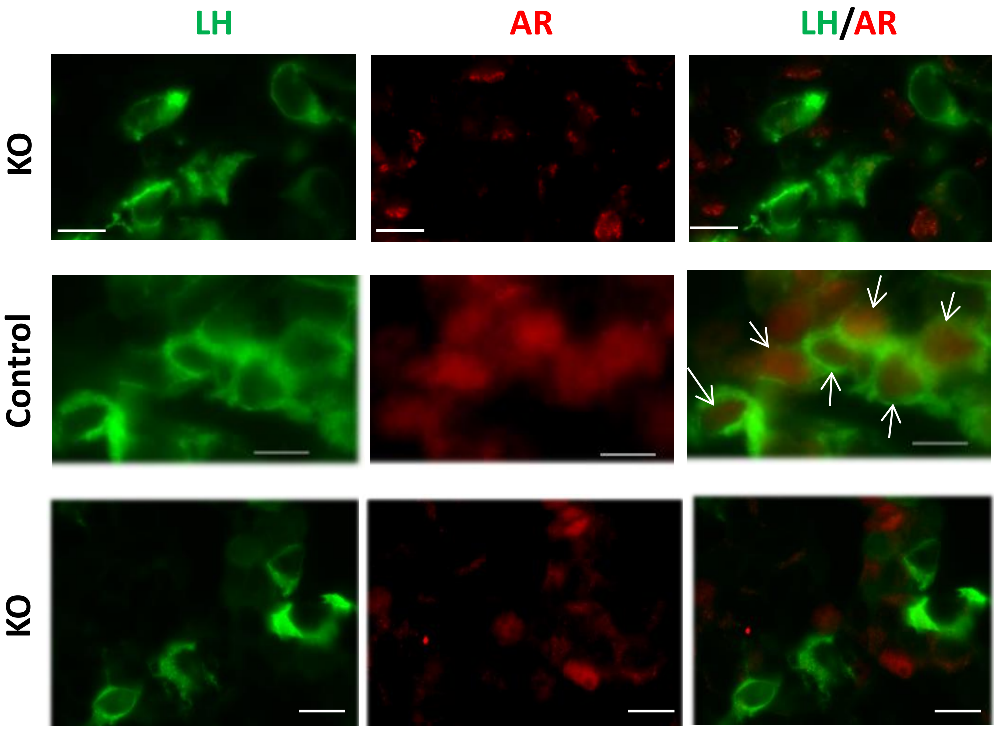

Protein standard marker
150
100
75
Pit-AR
50
35
25
Pit-actin
150
100
75
Ovary AR
50
35
Ovary actin
150
100
75
Liver-AR
50
35
Liver-actin
150
100
75
Hypo-AR
50
35
Hypo-actin

Suppl. Fig. 7



B



References

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