Supplementary Information

Functional analysis of rare anti-Müllerian hormone protein-altering variants identified in women with PCOS

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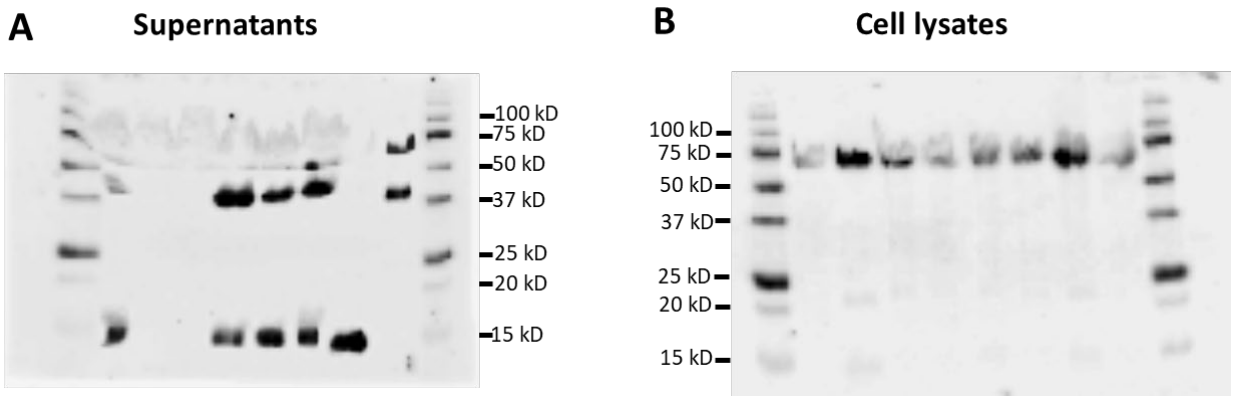
Supplementary Table SI Circulating AMH levels in individual carriers of six AMH variants that we did not functionally analyze.

Variants	A24T	P46A	T99S	R302Q	P366L	Splicing (ex2/3)
AMH, ng/mL*	9.43	3.49	12.23	5.99	27.52	7.43
AMH, ng/mL#	7.89	2.94	10.56	4.87	21.38	6.04

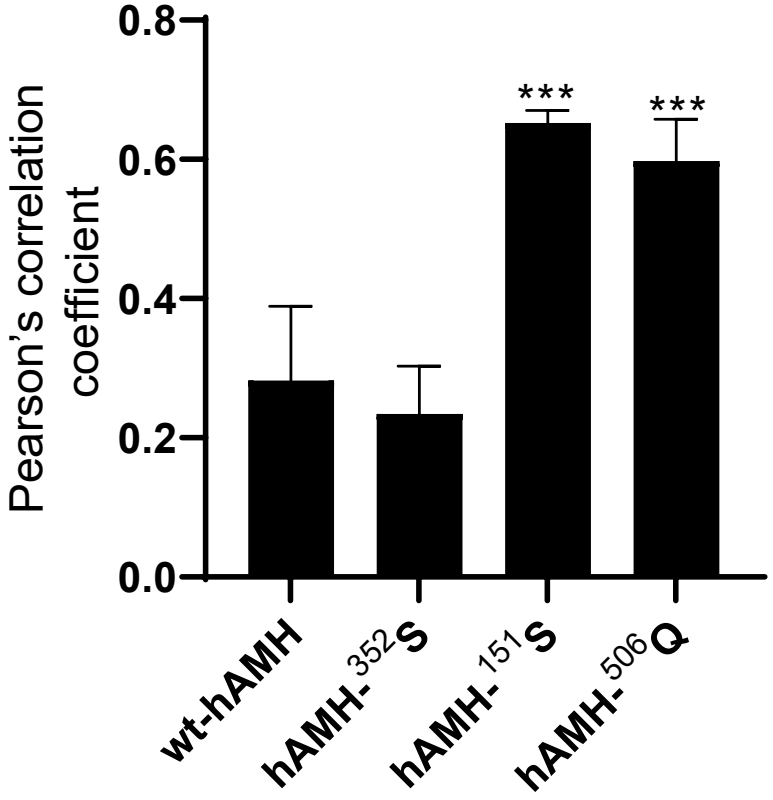
Measurement of serum AMH by * picoAMH assay (Ansh Labs) or # Lumipulse G1200 (Fujirebio).



Supplementary Figure S1. **Uncropped western blots of AMH variants in HEK293 cells.**

Western blot analysis of AMH variants: full uncropped blots of Figure 4.

Western blot analysis of the human embryonic kidney epithelial HEK293 cells stably expressing the anti-Müllerian hormone (AMH) variants with the wild-type cleavage site RAQR or the inactive cleavage site RAGA. The mature region-specific 5/6A antibody recognizes the AMH precursor protein (~75 kD), the cleaved C-terminal mature protein (~15 kD) and a second subunit owing to a possible second cleavage site (~40 kDa). The relative molecular masses (kD) of the protein marker are indicated. Supernatants **(A)** Full uncropped blot of Figure 4A. Lane 1: wt-hAMH-RAQR; lane 2: hAMH-^{151}S; lane 3: hAMH-^{506}Q; lane 4: hAMH-^{352}S; lane 5: hAMH-^{362}S; lane 6: hAMH-^{519}V; lane 7: wt-hAMH-RARR; lane 8: wt-hAMH-RAGA. Cell lysates **(B)** Full uncropped blot of Figure 4B. Lane 1: wt-hAMH-RAQR; lane 2: hAMH-^{151}S; lane 3: hAMH-^{506}Q; lane 4: hAMH-^{352}S; lane 5: hAMH-^{362}S; lane 6: hAMH-^{519}V; lane 7: wt-hAMH-RARR; lane 8: wt-hAMH-RAGA.



Supplementary Figure S2. Colocalization of wt-hAMH, hAMH-^{352}S, hAMH-^{151}S and hAMH-^{506}Q in stably transfected human embryonic kidney epithelial HEK293 cells.

Colocalization of wt-hAMH, hAMH-^{352}S, hAMH-^{151}S and hAMH-^{506}Q in stably transfected human embryonic kidney epithelial HEK293 cells with endoplasmic reticulum was analyzed by double immunofluorescent staining (confocal microscopy) and measured by Pearson's correlation coefficient (N=6). Statistical differences were analyzed by one-way ANOVA parametric test with Dunnett multi-comparison using the Prism 9 Software; ***P < 0.001