Figure S1. LR-AMH cleavage analysis. LR-AMH produced in CHO cells and recombinant AMH commercialized by Origen (cat. no. TP308397) (control AMH) were analyzed by western blotting with an anti-AMH antibody (cat. no. ab84952; Abcam) that recognizes the C-terminal domain. The results demonstrated that ~100% of the produced LR-AMH was in the cleaved form. Culture medium without FBS was used as the negative control. AMH, anti-Müllerian hormone; LR-AMH, active recombinant AMH.

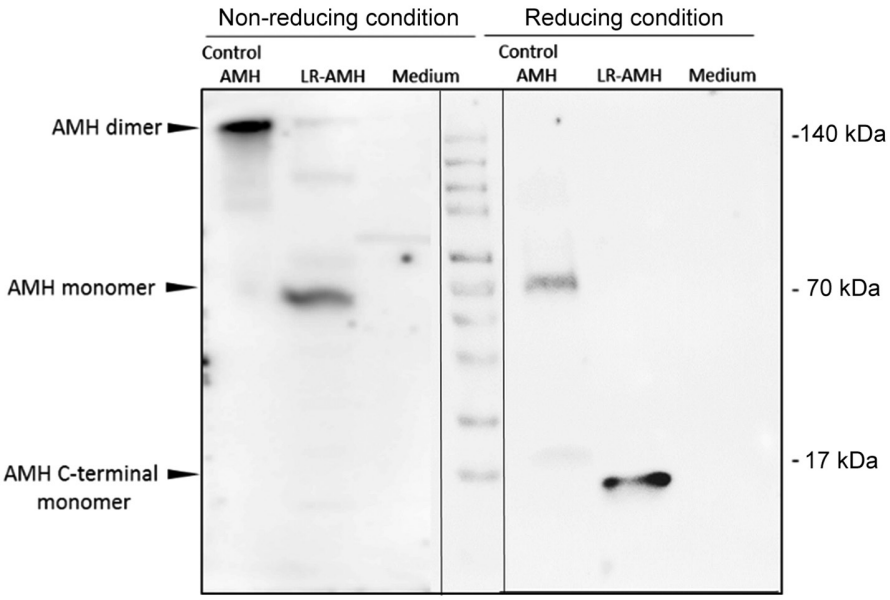

Figure S2. Design of the tetravalent BsAbs. Briefly, the anti-AMHRII-ALK2 and anti-AMHRII-ALK3 BsAbs were designed as follows: The anti-AMHRII VH/VL, 12G4 (grey) was inserted in position 2 of the BsAb construct (the furthest away from the Fc fragment) to ensure specific binding to its target. Anti-ALK2 (blue) and anti-ALK3 (red) VH/VL were inserted in position 1 of the BsAb construct, close to the Fc fragment. The mutations in yellow were introduced at the CH1/CL interface of the monoclonal antibody 1 to allow pairing of L1 and L2 to the fused H chain. BsAbs, bispecific antibodies; AMHRII, anti-Müllerian hormone type II receptor; ALK, activin receptor-like kinase; L, light chain; H, heavy chain; V, variable domain; C, constant domain.

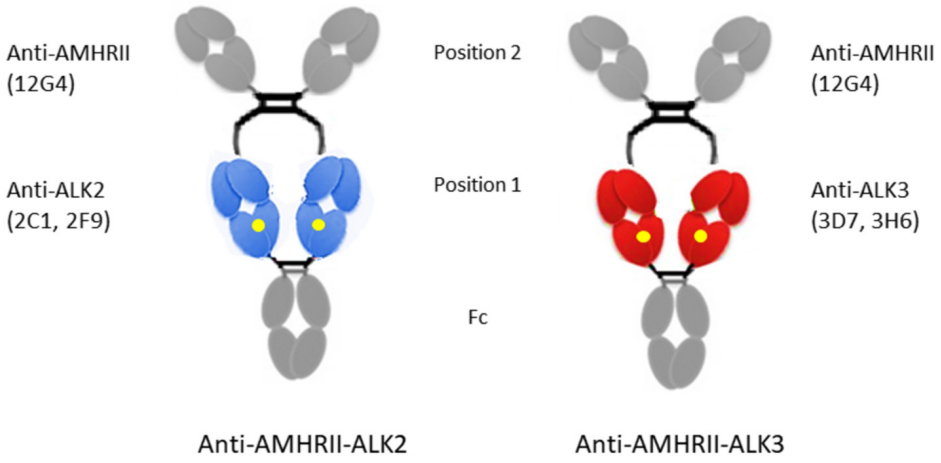

Figure S3. AMHRII expression validation in COV434-AMHRII and SKOV3-AMHRII cells. The efficacy AMHRII transfection was analyzed by western blotting in the COV434-AMHRII and SKOV3-AMHRII cell lines. AMHRII, anti-Müllerian hormone type II receptor; WT, wild-type.

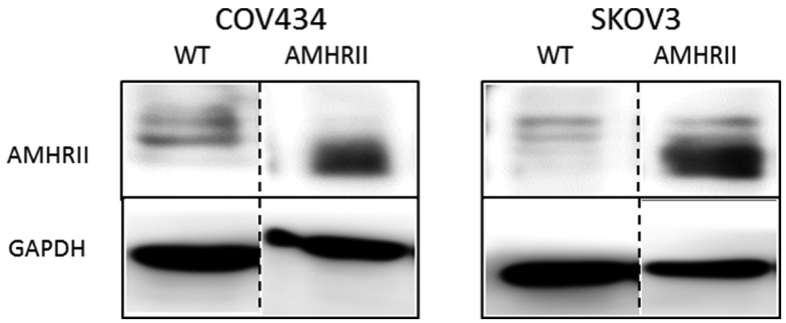

Figure S4. Flow cytometry analysis of apoptosis induced by AMH in COV434-AMHRII and SKOV3-AMHRII cells. Apoptosis was analyzed using by annexinV-7AAD assay following incubation with 25 nM active recombinant AMH for 24 h. Apoptosis, Annexin V-positive cells; late apoptosis, Annexin V/7AAD-positive cells; cell death, 7AAD-positive cells; AMH, anti-Müllerian hormone; AMHRII, AMH type II receptor.

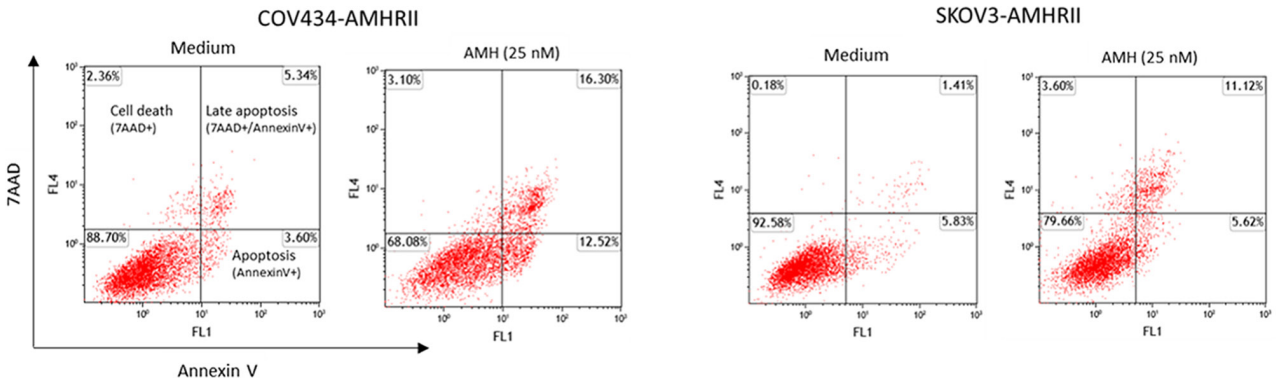

Figure S5. Anti-ALK2, -ALK3 and -ALK6 siRNA validation in COV434-AMHRII and SKOV3-AMHRII cells. The efficacy of the different siRNAs was analyzed by PCR and western blotting at 24 and 48 h post-transfection in the COV434-AMHRII and SKOV3-AMHRII cell lines. AMHRII, anti-Müllerian hormone type II receptor; ALK, activin receptor-like kinase; siRNA, si, small interfering RNA.

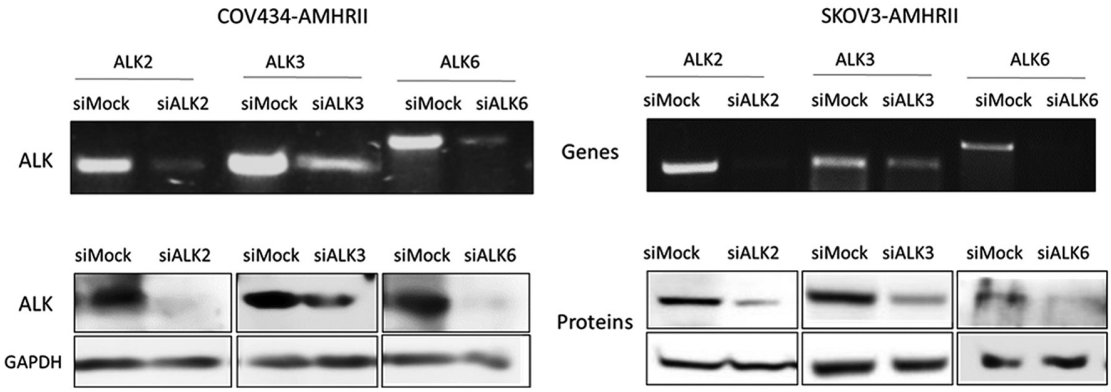

Figure S6. Differential expression of ALK2 and ALK3 following LR-AMH treatment is associated with the expression of cell survival and apoptosis markers in COV434-AMHRII, SKOV3-AMHRII, OVCAR8 and KGN ovarian cancer cells. Western blot analysis of AMH signaling (pSMAD1/5), ALK2 and ALK3 expression, apoptosis induction (cleaved caspase-3 and PARP) and pAKT levels following incubation with 1.6 and 25 nM LR-AMH for 6 h. AMH, anti-Müllerian hormone; LR-AMH, active recombinant AMH; AMHRII, AMH type II receptor; ALK, activin receptor-like kinase; p, phosphorylated; PARP, poly(ADP-ribose) polymerase.

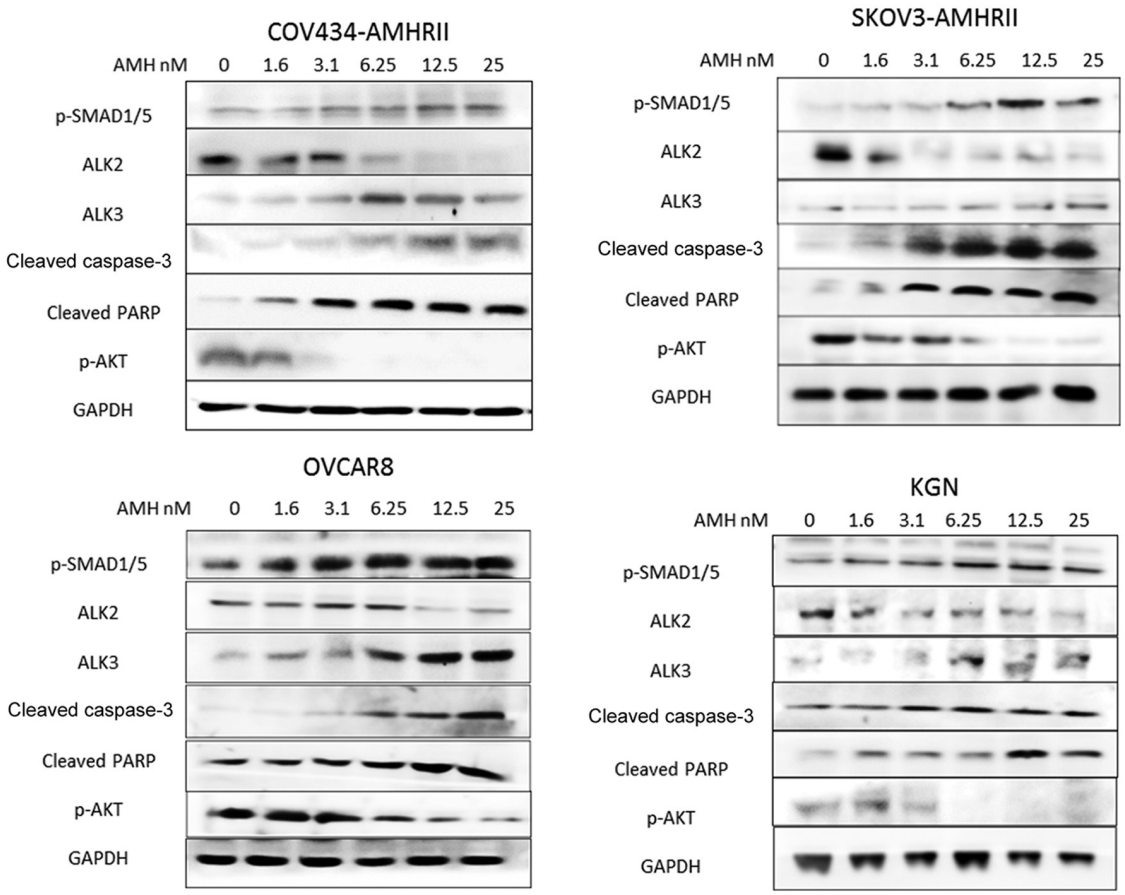

Figure S7. Differential expression of ALK2 and ALK3 following LR-AMH treatment is associated with the expression of cell survival and apoptosis markers in primary ovarian cancer cells. Western blot analysis of AMH signaling (pSMAD1/5), ALK2 and ALK3 expression, apoptosis induction (cleaved caspase-3 and PARP) and pAKT levels following incubation of cells isolated from ascites of three patients with ovarian cancer with 1.6 and 25 nM LR-AMH for 6 h. AMH, anti-Müllerian hormone; LR-AMH, active recombinant AMH; AMHRII, AMH type II receptor; ALK, activin receptor-like kinase; p, phosphorylated; PARP, poly(ADP-ribose) polymerase.