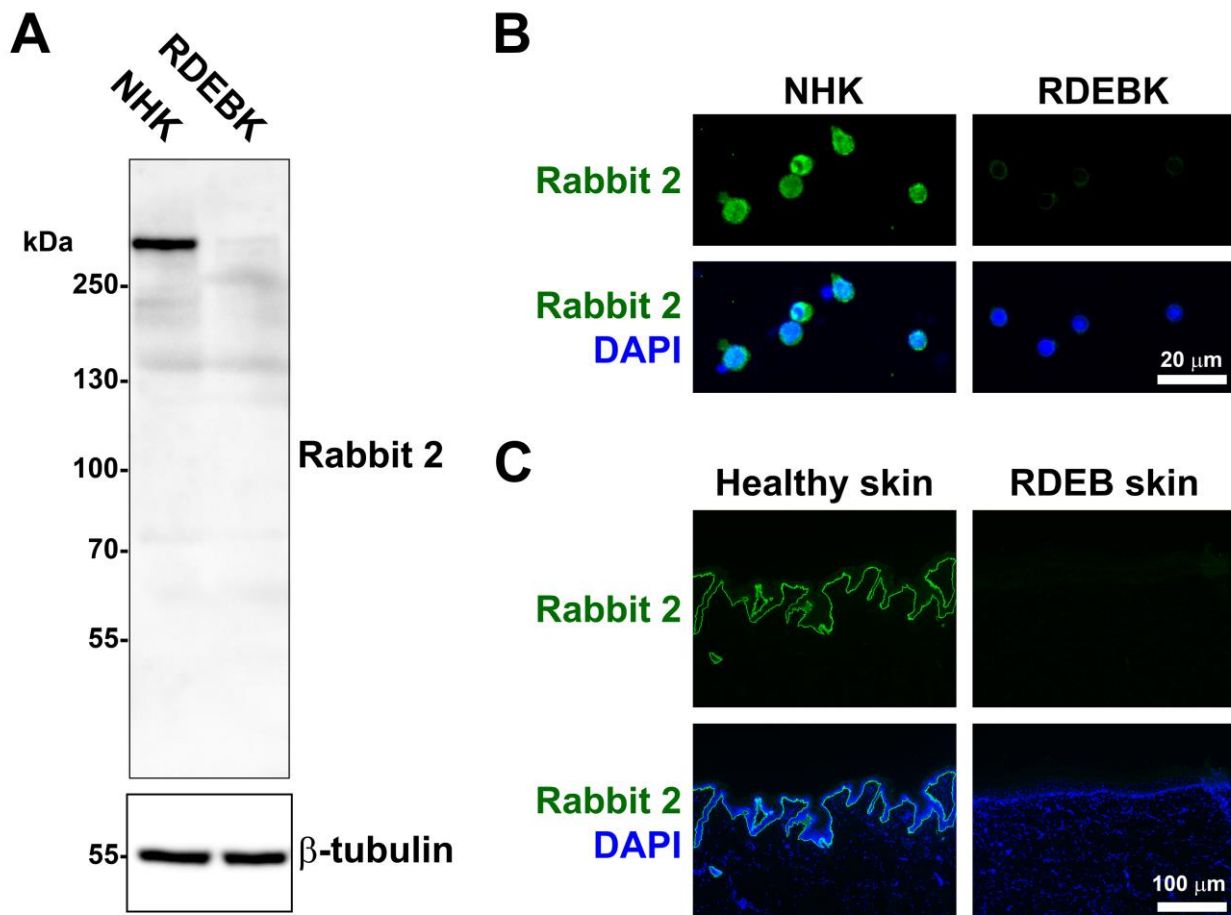**Supplementary material**

Generation of rabbit polyclonal human and murine collagen VII monospecific antibodies: A useful tool for dystrophic epidermolysis bullosa therapy studies

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Supplemental Fig. 1. Antiserum from rabbit 2 immunized with hLH 7:2 specifically recognizes collagen VII. A, Western blots of protein lysates from normal human keratinocytes or keratinocytes from donors with complete collagen VII deficient RDEB probed with rabbit 2 serum or β-tubulin used to ensure equal loading. **B**, Staining of keratinocytes as in A with rabbit 2 serum. Nuclei counterstained with DAPI, scale bar = 20 μm. **C**, Staining of human skin cryosections from healthy donors or donors with complete collagen VII deficient RDEB with rabbit 2 serum. Nuclei counterstained with DAPI, scale bar = 100 μm.
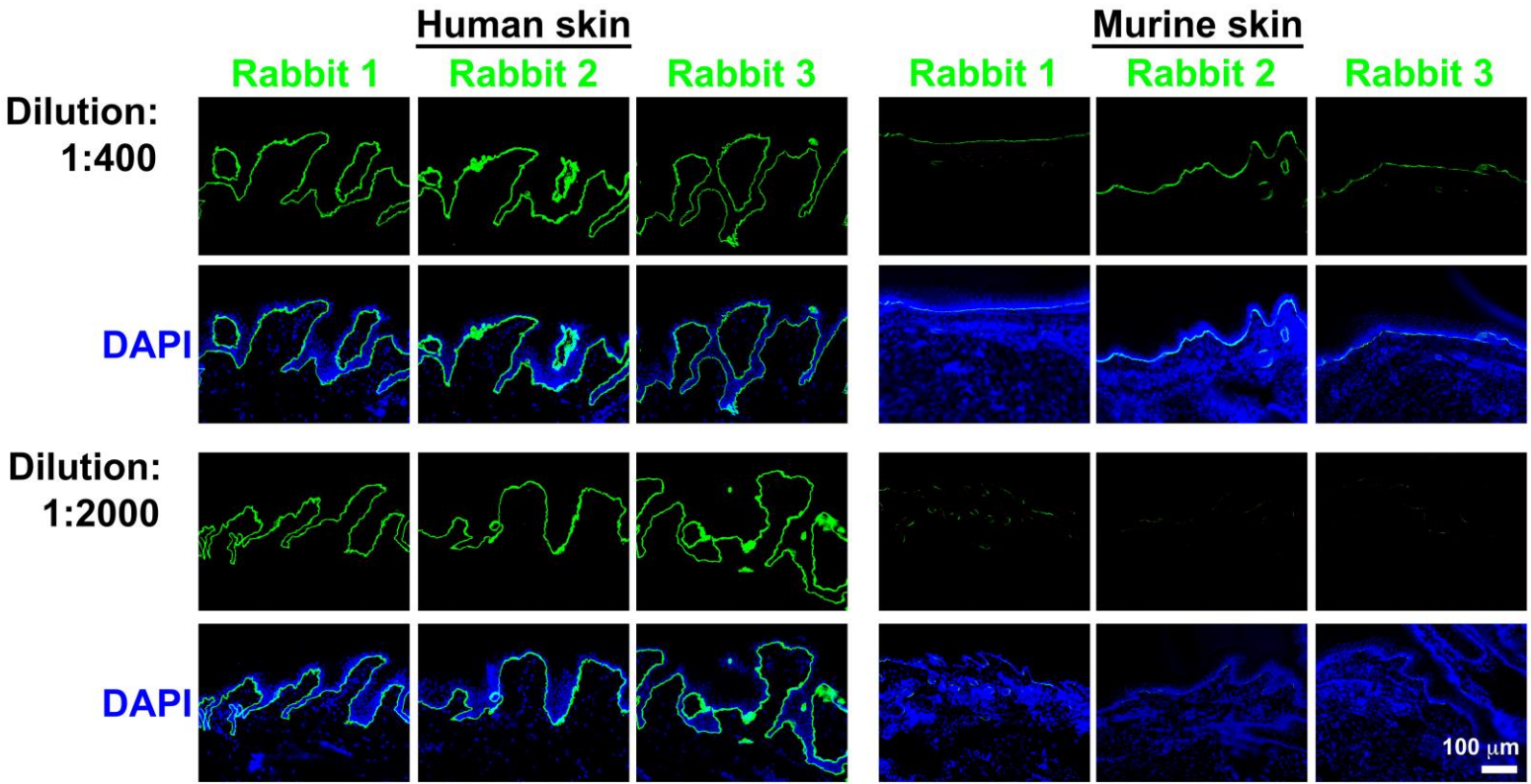
Supplemental Fig. 2. Sera from rabbits immunized with the hLH 7:2 strongly recognize human but minimally murine collagen VII at higher dilutions. Human and murine wild-type skin cryosections stained with sera from rabbits 1 – 3 immunized with the hLH 7:2 peptide at 1:400 or 1:2000 dilutions. Nuclei counterstained with DAPI, scale bar = 100 μm.
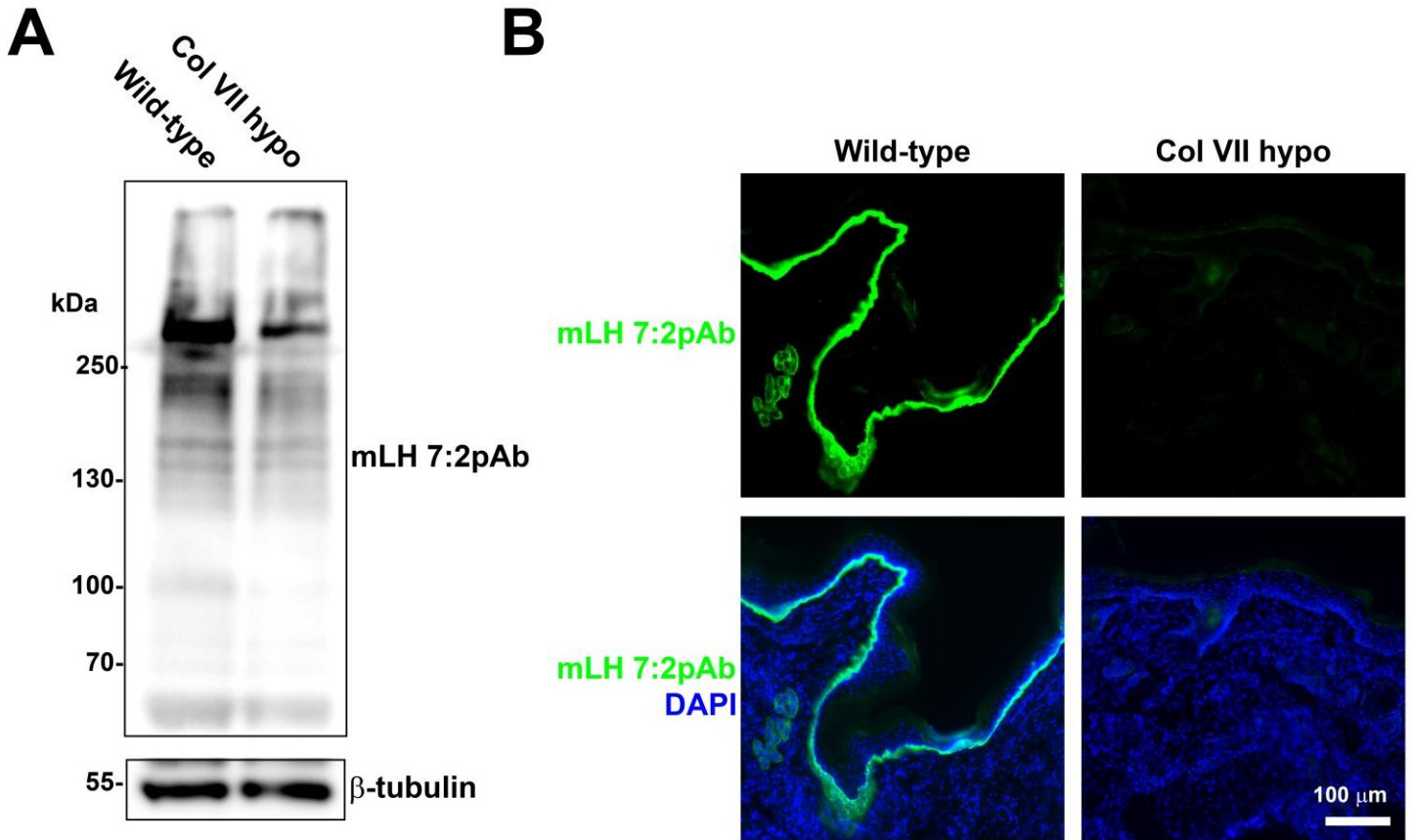
Supplemental Fig. 3. mLH 7:2pAb specifically recognizes collagen VII. A, Western blot of skin lysates from wild-type and collagen VII hypomorphic mice probed with mLH 7:2pAb or β-tubulin used to ensure equal loading. **B**, Skin cryosections from mice as in A stained with mLH 7:2pAb (green). Nuclei counterstained with 4′, 6-diamidino-2-phenylindole (DAPI), scale bar = 100 μm.
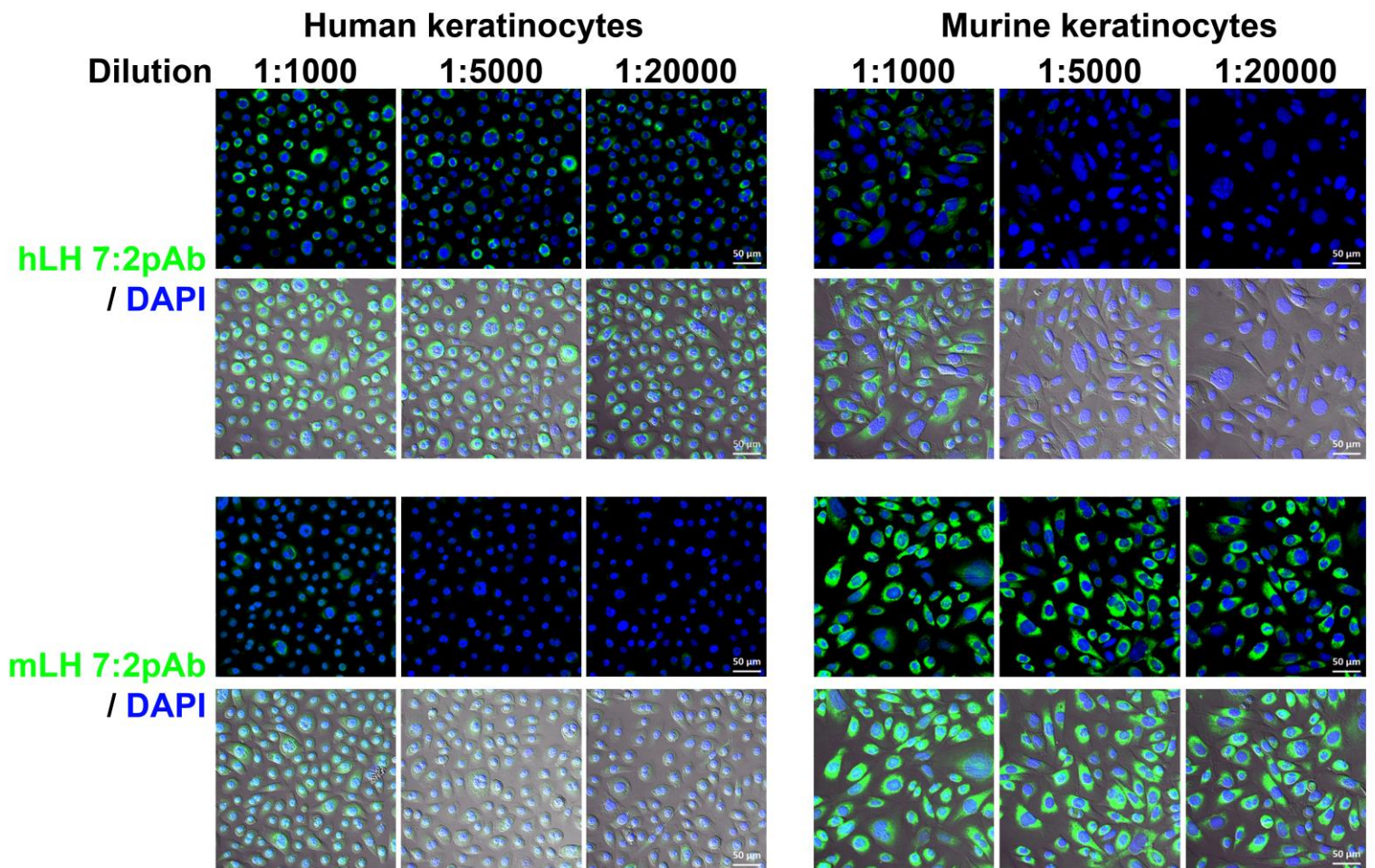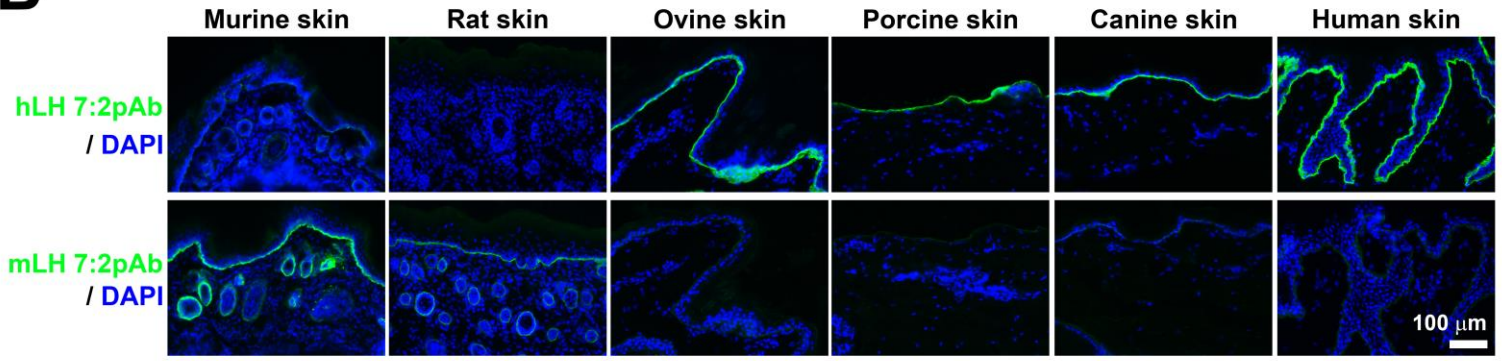
Supplemental Fig. 4 mLH 7:2pAb strongly recognizes murine but minimally human collagen VII at higher dilutions. Human or murine wild-type keratinocytes stained with hLH 7:2pAb and mLH 7:2pAb at the indicated dilutions. Nuclei counterstained with DAPI, scale bar = 50 μm.
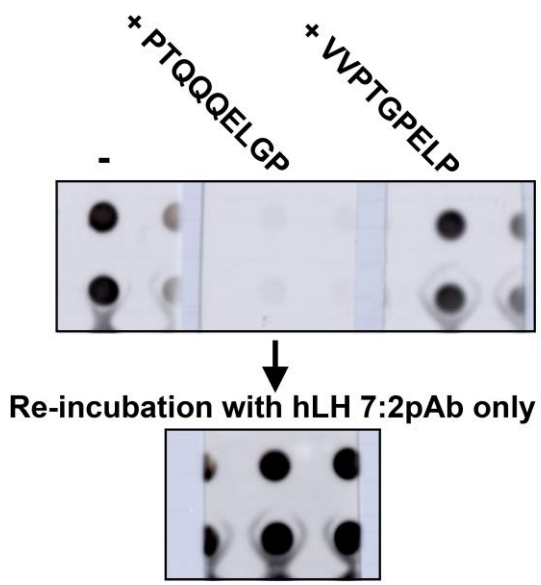
A

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Murine LH 7:2   LSLQNITSHSLLVAWRRVPGANGYRVTWRDLSGGPTQQQDLSPGQGSVFLDHLEPGTDYE   60
Rat LH7:2       LSVQNITSHSLLVAWRRVPGANGYRVTWRDLSGGAQQQDLSPGQGSVFLDHLKPGTDYE    60
Ovine LH 7:2    VTIQNTTAHSLLVAWRSVPGATGYRVTWRVVRGGATQQQELGPGQGSVLLRDLQPGTDYE   60
Porcine LH 7:2  LTIQNSTAHSFLVAWQSVPGATGYRVTWRVLSGGAMQQQELGPGQGSVLLHDLEPGTDYE   60
Human LH 7:2    LTIQNTTAHSLLVAWRSVPGATGYRVTWRVLSGGPTQQQELGPGQGSVLLRDLEPGTDYE   60
Canine LH 7:2   LTIQNTTTHSLLVAWRSVPGATGYRVTWRVLSGGVTQQQELGPGQGSVLLRDLEPG-DYE  59
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Murine LH 7:2   VTVSALFGHSVGPAASLTARTASSVEQTLHPIILSPTSILLSWNLVPEARGYRLEWRRES  120
Rat LH7:2       VTVSALFGRSVGPAASLTTRTASSVEQTLRPIILSPTSILLSWNLVPEARGYRLEWHRES  120
Ovine LH 7:2    VTVSALLGRSVGPATSLTARTDTSVEQTLRPVILGPTSILLSWNLVPEARGYRLEWRRES  120
Porcine LH 7:2  VTVSALFGRNLGPATSLTARTDISVEQTLRPLILGPTSILLSWNLVPEARGYRLEWRRES  120
Human LH 7:2    VTVSTLFGRSVGPATSLMARTDASVEQTLRPVILGPTSILLSWNLVPEARGYRLEWRRET  120
Canine LH 7:2   VTVSSLLGRSIGSASSLTARTDTSVEQTLRPVILGPTSILVSWNLVPEARGYRLEWRRES  119
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Murine LH 7:2   GLETPQKVELPPDVTRHQLDGLQPGTEYRLTYTLLEGREVATPATVVPTGLE--QLVSP  178
Rat LH7:2       GLEPPQKVELLPDVTRYQLDGLQPGTEYRLTYTLLEGREVATPATVVPTGLE--QLVGP  178
Ovine LH 7:2    GLEVPQKVVLPSDVTRYQLDGLQPGTEYRLTYTLLEGREVATPATVVPTGEGPRGPEGP  180
Porcine LH 7:2  GLEVPQKVVLPSDVTHYQLDGLQPGTEYRLTYTLLEGREVATPATVVPTG--PELPVGP  178
Human LH 7:2    GLEPPQKVVLPSDVTRYQLDGLQPGTEYRLTYTLLEGHEVATPATVVPTG--PELPVSP  178
Canine LH 7:2   GLGVPQKVVLPSDVTRYQLDGLQPGTEYRLTYTLLEGREVATPATVVPTG--PELPVGP  177
                **  **** *  ***  ********************** ***********          *

Murine LH 7:2   VMNLQAIELPGQRVRVSWNPVPGATEYRFTVRTTQGVERTLLLPGSQTTFDLDDVRAGLS  238
Rat LH7:2       VMNLQATELPGQRLRVSWNPVLGATEYRFTVRTPQGVERTLLLPGSQTTFDLDDVRAGIS  238
Ovine LH 7:2    VTALQATELPGQRVRVSWSPVPSATEYRITVRSAQGVERSLVLPGSQTAFDLDDVRAGLS  240
Porcine LH 7:2  VMDLQATELPGQRVRVSWSPVPSATEYRITMRSTQGVERTLVLPGSQTAFDLDDVRAGLS  238
Human LH 7:2    VTDLQATELPGQRVRVSWSPVPGATQYRIIVRSTQGVERTLVLPGSQTAFDLDDVQAGLS  238
Canine LH 7:2   VTDLQATELPGQRVRVSWSPVPGATEYRITVRSAQGVERTLVLPGSQTAFDLDDVRAGLS  237
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Murine LH 7:2   YTVRVSAR  246
Rat LH7:2       YTVRVSAR  246
Ovine LH 7:2    YTVRVSAR  248
Porcine LH 7:2  YTVRVSAR  246
Human LH 7:2    YTVRVSAR  246
Canine LH 7:2   YTVRVSAR  245
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B

| Murine skin | Rat skin | Ovine skin | Porcine skin | Canine skin | Human skin |

hLH 7:2pAb / DAPI

mLH 7:2pAb / DAPI

100 μm

C

-     + PTQQQELGP   + VVPTGPELP

↓

Re-incubation with hLH 7:2pAb only

Supplemental Fig. 5 Species specificity of mLH 7:2pAb and hLH 7:2pAb and identification of one epitope predominantly recognized by hLH 7:2pAb. A, Alignment of murine, rat, ovine, porcine, canine and human LH 7:2 peptides. Amino acids in the predicted N-terminal predominant immunoepitope shared by all species are marked in yellow, amino acids common for rat and mouse in red and amino acids shared by sheep, pig, dog and human in blue. **B**, Staining of skin from species as indicated with mLH 7:2pAb (green) and hLH 7:2pAb at 1:2000 dilution (green). Nuclei counterstained with DAPI (blue**),** scale bar = 100 μm. **C**, Peptide competition binding assay**.** hLH 7:2pAb incubated with or without the indicated peptides (0.1 mg/ml) and then incubated with membranes on which 100 ng recombinant human collagen VII had been immobilized. The results show that the PTQQQELGP peptide blocks binding of the antisera to human collagen VII. The almost complete absence of signal is not due to insufficient amounts of collagen VII on the membrane as re-incubation with hLH 7:2pAb only results in a strong signal.