

## Supplementary Data

| VHH       | FR1                              | CDR1            | FR2                         | CDR2           |
|-----------|----------------------------------|-----------------|-----------------------------|----------------|
|           | ←-----→                          | ←-----→         | ←-----→                     | ←-----→        |
| A7        | EVQLVESGGGLVQAGGSLRSLCAAS        | GSIVTFNP        | MGWYRQAPGNQ--RELVASI        | TSGG-GA        |
| B7        | EVQLVESGGGLVQAGGSLRLCAAS         | GRTFSNYR        | MGWFRQAPGQE--REFVAAI        | SGSGSFT        |
| B12       | EVQLVESGGGLVQPGGSLRSLCAAS        | GFTFSSYA        | MSWVRQAPGKG--LEWVSAI        | NSGGGST        |
| C9        | EVQLVESGGGLVQAGGSLRSLCAAS        | GFTISNYG        | MSWVRQAPGKGPEWVSAI          | NSGGDST        |
| D4        | EVQLVESGGGLVQAGDSLRLCAAS         | GRSISLYA        | MAWFRQAAGKE--REFVAAI        | NWSGGST        |
| <b>G5</b> | <b>EVQLVESGGGLVQAGGSLRSLCAAS</b> | <b>GRALSRSP</b> | <b>MAWFRQAPGKE--REFVHW</b>  | <b>ISG--ST</b> |
| <b>H7</b> | <b>EVQLVESGGGLVQAGGSLRSLCAAS</b> | <b>G---STGA</b> | <b>MAWFRQAPGKE--RDLVASI</b> | <b>SRSGVST</b> |

  

| VHH       | FR3   | CDR3                         | FR4                |
|-----------|---|------------------------------|--------------------|
|           | ←-----→   | ←-----→                      | ←-----→            |
| A7        | NYVDSVKGRFTISVDSAKNTVYLMNSLKPEDTAVYYCNA         | D-----IFSSSRLSWDNY           | WGQGTQVTVSS        |
| B7        | YYADSVKGRSTISRDNAKNTVYLMNSLKPEDTAVYYCGA         | G-----VHLGAATSYTRYD-F        | WGQGTQVTVSS        |
| B12       | SYADSVKGRFTISRDNAKNTLYLMNSLKPEDTAVYRCAK         | ---YYEADP---AKNEYD-Y         | WGQGTQVTVSS        |
| C9        | RYADSVKGRFTISRDNAKNTLYLMNSLKPEDAAYVFCTR         | EKTAYYCSGSGCYDPRYEFD-Y       | WGRGTQVTVSS        |
| D4        | RYADSVKGRFISRDTAKNTVYLTMNSLKPEDTAVYYCAT         | DSS---TTVVFYSSNSLR-Y         | WGQGTQVTVSS        |
| <b>G5</b> | <b>YYADSVKGRFTISRDNAEENTVYLMNSLKPEDTAVYYCAA</b> | <b>GFA---PDTPSIFTSPTYY-Y</b> | <b>WGQGTQVTVSS</b> |
| <b>H7</b> | <b>YYADSVKVRFTISRDNAKNTVFLQMNLLKPEDTGVYYCAA</b> | <b>G-----PTFRQSRATYT-D</b>   | <b>WGQGTQVTVSS</b> |

**SUPPLEMENTARY FIG. S1.** Amino acid sequence alignment of VHH targeting DKK1 derived from a llama immunized library. Sequencing of the 14 isolated clones resulted in the identification of 7 unique clones. The sequence numbering is according to Chothia [1] where according to whom the different FR and CDR are identified. The sequences of G5 and H7 are highlighted in *bold*. CDR, complementary-determining region; DKK1, Dickkopf 1 homolog; FR, framework residues.