Introduction:
Since decades rabbits are used for generation of antibodies and are known to produce a great diversity of high affinity antibodies even against difficult targets. Humanization of rabbit antibodies, however, is considered to be problematic due to several structural constraints.
We developed a general approach that allows humanization of rabbit derived antibodies and enables mass humanization of entire rabbit immune repertoires.

Figure 1. Humanization landscapes are prepared to accept rabbit-derived SDRs:
Transfer principle specificity determining elements

Figure 2. Mass humanization landscapes represent the intermediate average of all possible humanizations:

Figure 3. Each H3-defined clone can undergo tens of thousands of successful humanizations

Figure 4. Workflow:
Immunize rabbits
PCR amplify SDRs
Clone rabbit SDRs in humanization landscape
Phage Panning: 2-3 cycles
Hit identification: ELISA
Sequence analysis of hits

Figure 5. Method delivered many hits after 2 panning rounds
ELISA with soluble scFv after 2 panning rounds

Figure 6. 21 CDR-H3 groups identified
Hit identification: ELISA

Figure 7. Imprint of in vivo affinity maturation detectable in CDR-H3 sequences:

Conclusion:
- Mass humanization of rabbit antibodies delivers multiple humanized antigen-specific hits
- Highly potent method to isolate humanized antibodies from rabbit immune repertoires

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