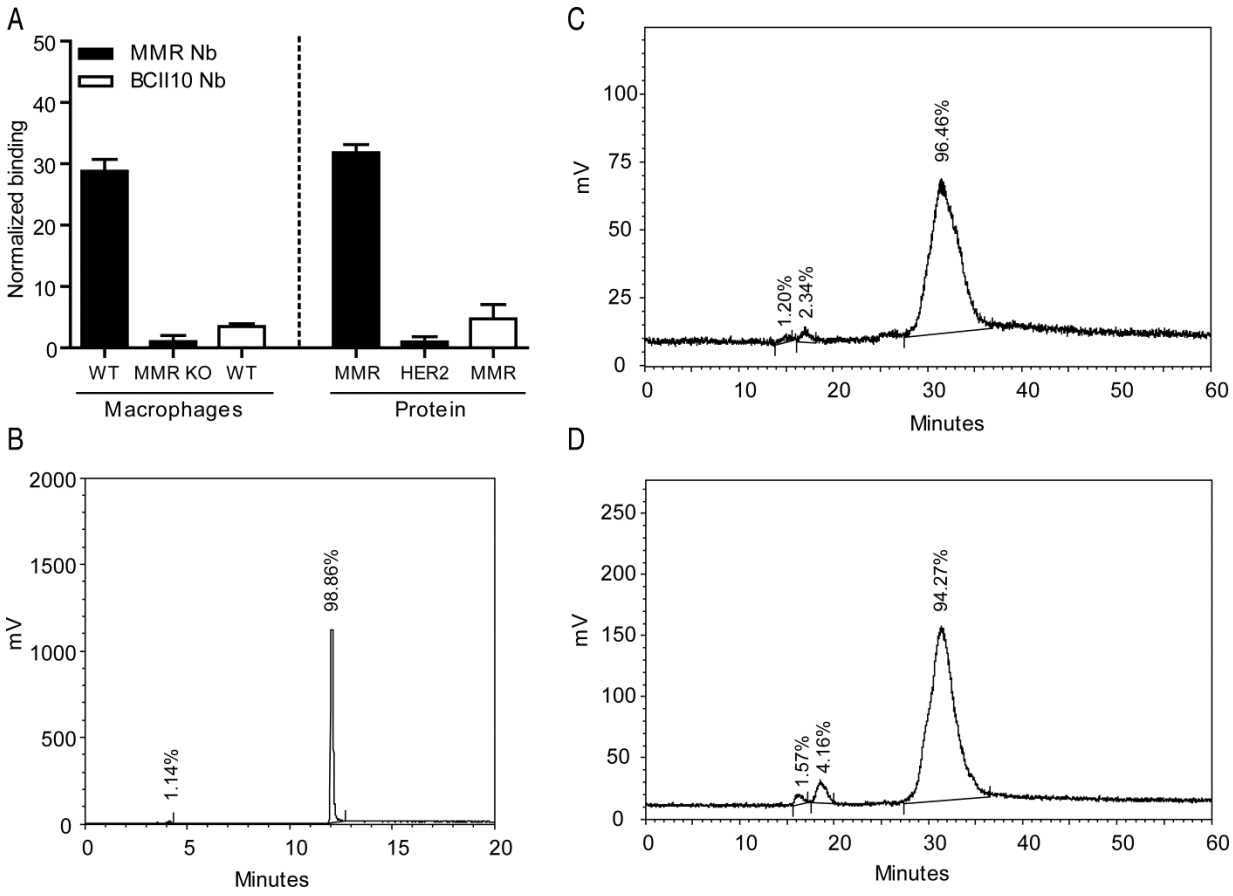
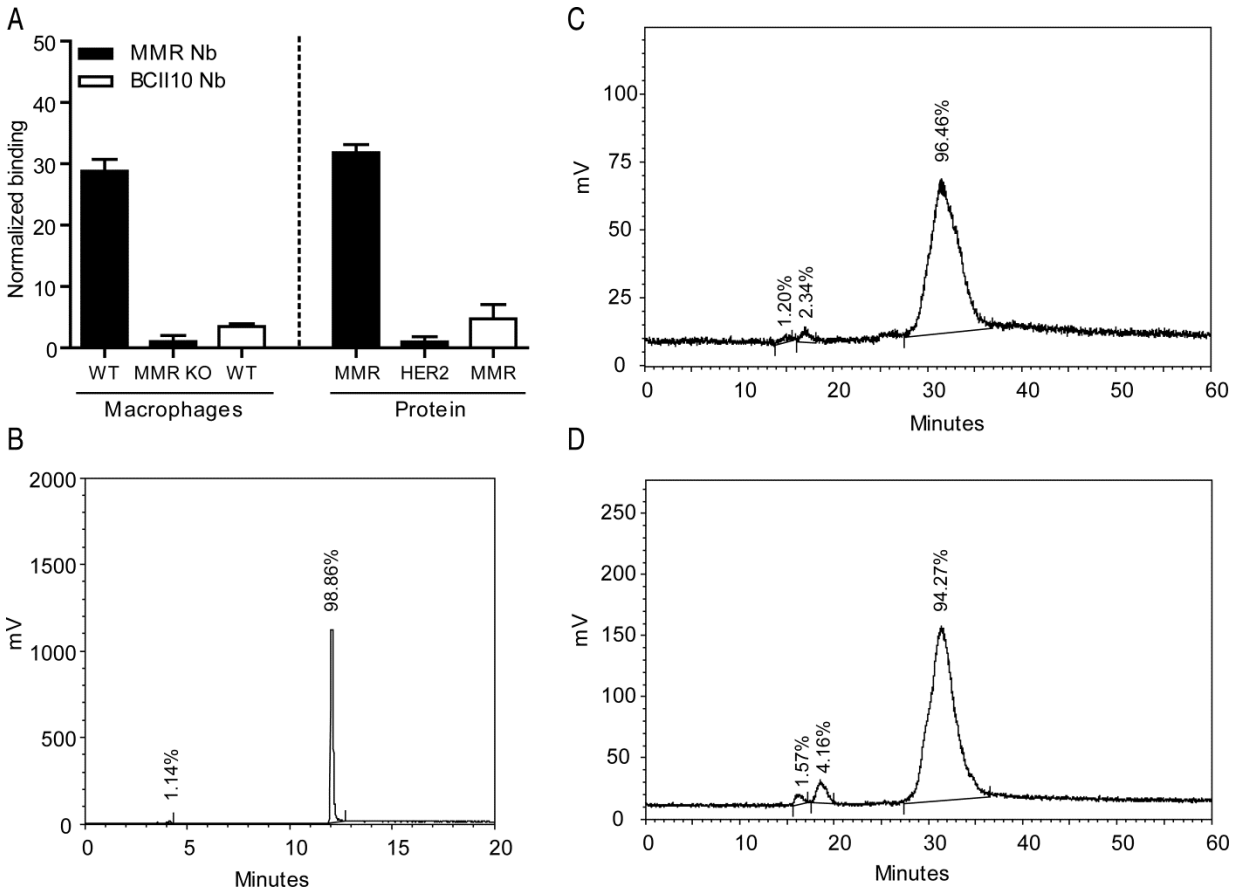

A
MMR Nb
BCII10 Nb
Normalized binding
WT
MMR KO
WT
MMR
HER2
MMR
Macrophages
Protein
B
mV
98.86%
1.14%
Minutes
C
mV
96.46%
1.20%
2.34%
Minutes
D
mV
94.27%
1.57%
4.16%
Minutes



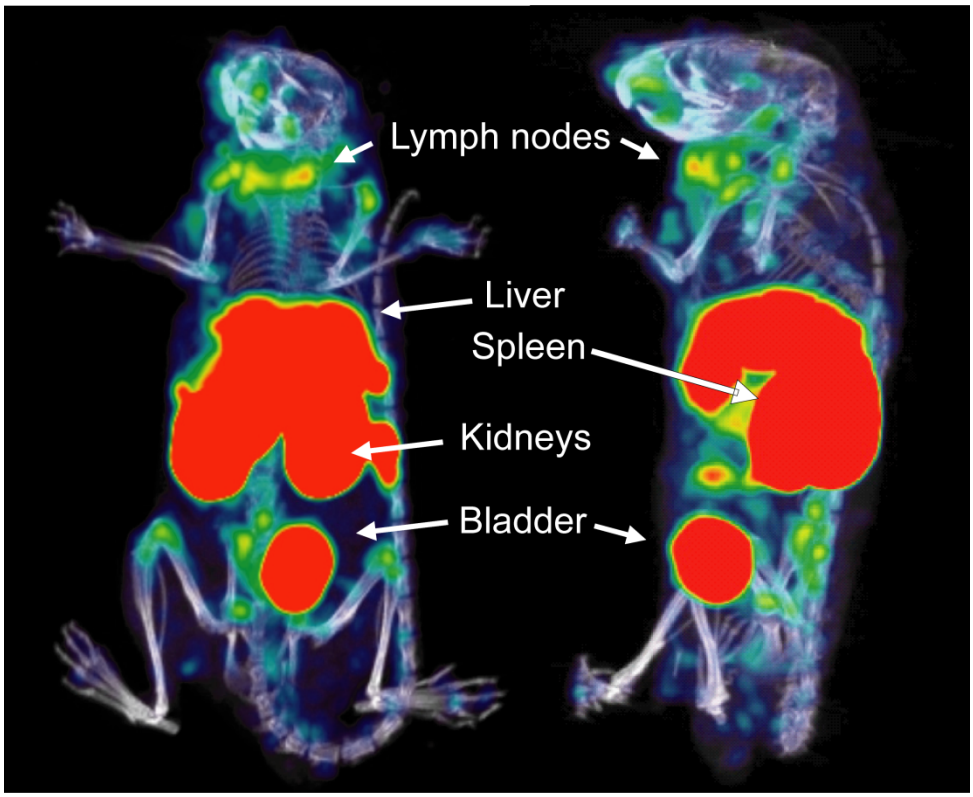

**Supplemental figure 1: Binding specificity, purity and stability of radiolabeled nanobodies.**

**A:** *In vitro* binding specificity of $^{99m}$Tc-labeled nanobodies was investigated by quantification of the binding of the nanobodies (10 nM) to MMR-expressing peritoneal macrophages from wildtype mice and MMR knockout (KO) mice. Binding of the BCII10 nanobody to wildtype macrophages was included as a control. Binding of the MMR nanobodies to recombinant MMR or HER2 (control) protein further confirmed the specificity of the nanobodies. Graph shows mean of 3-4 normalized values +SEM.

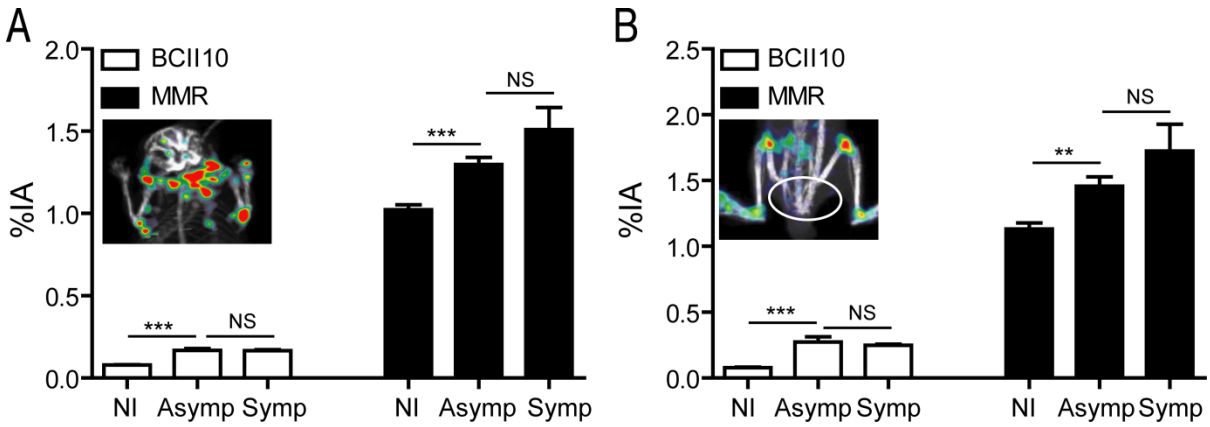
**B:** Radiochemical purity was assessed by performing RP-HPLC on a PLRP-S column using a gradient of 75% 0.1%TFA in $H_20$ / 25% acetonitrile to 100% acetonitrile at 1 ml/mm. The resulting radioactive signal shows a radiochemical purity of almost 99%.

**C:** Gel filtration of the $^{99m}$Tc-labeled nanobodies using a Superdex 75 column resulted in a peak of >96%.

**D:** Incubation of the radiolabeled nanobodies in mouse serum for three hours at 37°C (comparable to the in vivo situation) resulted in a similar profile as observed in panel C and confirmed the stability of the nanobodies.




**Supplemental figure** 2: SPECT imaging was performed after micro-CT scans on healthy animals at 3 hours post injection and shows the biodistribution of injected MMR nanobody.




**Supplemental figure 3:** The radioactivity emitted by each area of interest [lymph nodes (A), immunization site (B)] was quantified using AMIDE software as % of injected activity (%IA) in groups of nonimmunized mice (NI), immunized mice without symptoms (Asymp) and with symptoms of arthritis (Symp). Inset images show quantified areas. Bars represent means of 6 to 12 values (NI: n=9; Asymp: n=6; Symp: n=12) ± SEM (*p<001 **p<0 0001 Mann-Whitney's U test). NS: no significant difference.

**Supplemental table 1: Quantification of $^{99m}$Tc-labeled BCII10 or MMR nanobody accumulation.**

| | BCII10 | | | | MMR | | | |
|---|---|---|---|---|---|---|---|---|
| | NI | Asymp | Symp | | NI | Asymp | Symp | |
| | | | NA | A | | | NA | A |
| **Lymph nodes (%IA)** | 0.08 ± 0.003 | 0.17 ± 0.011 | 0.17 ± 0.007 | | 1.02 ± 0.030 | 1.30 ± 0.044 | 1.51 ± 0.13 | |
| **Immunization site (%IA)** | 0.08 ± 0.005 | 0.27 ± 0.039 | 0.25 ± 0.0078 | | 1.13 ± 0.046 | 1.46 ± 0.071 | 1.73 ± 0.20 | |
| **Ankles (%IA)** | 0.007 ± 0.0008 | 0.021 ± 0.002 | 0.018 ± 0.002 | 0.073 ± 0.007 | 0.063 ± 0.003 | 0.074 ± 0.004 | 0.06 ± 0.003 | 0.36 ± 0.03 |
| **Metatarsal joints (%IA)** | 0.0028 ± 0.0005 | 0.013 ± 0.001 | 0.0093 ± 0.002 | 0.038 ± 0.004 | 0.062 ± 0.003 | 0.05 ± 0.003 | 0.060 ± 0.004 | 0.20 ± 0.03 |
| **Knees (%IA)** | 0.016 ± 0.0009 | 0.053 ± 0.007 | 0.059 ± 0.01 | 0.087 ± 0.003 | 0.30 ± 0.01 | 0.29 ± 0.01 | 0.35 ± 0.03 | 0.56 ± 0.06 |
| **Dissected paws (%IA/g)** | 0.13 ± 0.006 | 0.40 ± 0.05 | 0.33 ± 0.02 | 0.70 ± 0.05 | 1.67 ± 0.1 | 1.88 ± 0.2 | 3.03 ± 0.4 | 4.83 ± 0.2 |

Table shows the mean values (%IA or %IA/g) obtained from the analysis shown in figure 5 ± SEM. Mice were divided into three groups. NI: non-immunized mice; Asymp: immunized mice without symptoms; Symp: immunized mice with symptoms of arthritis. Since collagen-induced arthritis is not a symmetric disease and limbs in the same mouse can have different scores, the ankles, metatarsal joints, knees and dissected paws of Symp mice were further subdivided in NA (non-arthritic, no scores) or A (arthritic, with scores).