

1. Summary	v
2. Zusammenfassung	vii
3. Introduction	1
3.1 Skin structure and function	1
3.1.1 Epidermis.....	1
3.1.2 Dermis.....	3
3.1.3 Hypodermis	4
3.1.4 Differences between human and mouse skin	4
3.2 Immunity and keratinocytes	5
3.2.1 Receptors	5
3.2.2 Cytokines and chemokines	6
3.2.3 Antimicrobial peptides.....	7
3.3 Immunity and skin myeloid cells: Monocytes/macrophages, neutrophils, and Langerhans cells.....	7
3.3.1. Neutrophils	8
3.3.2 Monocytes/dermal macrophages.....	8
3.3.3 Langerhans cells.....	8
3.4 UVR interaction with the skin.....	9
3.4.1 UVB immunomodulation/inflammation.....	11
3.4.2 Molecular mechanisms of UV-induced apoptosis	12
3.4.3 UV radiation: Beneficial effects.....	13
3.5 Wound healing.....	13
3.5.1 The inflammation phase.....	15
3.5.2 The proliferation phase	15
3.5.3 The tissue re-modeling phase	17
3.6 NF-E2-related factor transcription factor family members	17
3.6.1 NFE2-related factors (Nrf1, Nrf2 and Nrf3) family – structural features.....	18
3.6.2 Expression and function of NF-E2-related factors	19
3.6.2.1 Nrf1	19
3.6.2.2 Nrf2	19
3.6.3.3 Nrf3	22
3.7 Aim of the PhD thesis	23
3.7.1 Project 1	24
3.7.2 Project 2	24
3.7.3 Project 3	24
3.8 References.....	25
4. Projects	45
4.1 Nrf2 is highly expressed in neutrophils, but myeloid cell-derived Nrf2 is dispensable for wound healing in mice	45
4.2 Role of Nrf2 in myeloid cells and keratinocytes in the UVB response of the skin.....	69
Abstract.....	71
Introduction	71
Materials and Methods	73
Results	78
Discussion.....	92
Acknowledgement	94
References	94

4.3 Kinetics of myeloid cells in skin during wound healing.....	99
Abstract.....	101
Introduction	101
Materials and Methods	103
Results	107
Discussion.....	116
References.....	118
4.4 Regulatory T cells are required for normal and activin promoted wound repair in mice.....	123
5. General discussion and outlook.....	145
5.1 Role of myeloid-derived Nrf2 in wound healing.....	146
5.2 Role of Nrf2 in myeloid cells and keratinocytes in the UVB response of the skin.....	147
5.3 Kinetics of myeloid cells in skin during wound healing.....	150
6 MATERIALS & METHODS.....	161
6.1 Materials.....	161
6.1.1 Chemicals and solutions.....	161
6.1.2 Enzymes, reagents and kits	162
6.1.3 Cell culture media, additives and other materials.....	163
6.1.4 Plastic ware, glassware, consumables	164
6.1.5 Laboratory Equipment.....	164
6.1.6 Software.....	165
6.1.7 Buffers and solutions	165
6.1.8 Antibodies.....	168
6.1.9 Primers.....	170
6.2 Methods.....	172
6.2.1 Animal Experiments.....	172
6.2.1.1 Genetically modified mice.....	172
6.2.1.2 Mouse genotyping.....	173
6.2.1.3 Wounding.....	175
6.2.1.4 UV irradiation	175
6.2.2 Histology and immunostaining methods	175
6.2.2.1 PFA-fixed and acetic ethanol-fixed samples.....	175
6.2.2.2 Deparaffinization	177
6.2.2.3 Haematoxylin/Eosin staining.....	177
6.2.2.4 Herovici staining.....	178
6.2.2.5 Cryosections.....	180
6.2.2.6 Cleaved Caspase 3 staining	180
6.2.2.7 Microscopy	180
6.2.3 RNA methods	181
6.2.3.1 RNA extraction from wounds/tissue, cells and sorted cells	181
6.2.3.2 Determination of RNA concentration and quality control	181
6.2.3.3 cDNA synthesis.....	182
6.2.4 Cell culture studies.....	183
6.2.4.1 Isolation of primary murine keratinocytes.....	183
6.2.4.2 UVB irradiation of primary murine keratinocytes.....	184
6.2.4.3 Determination of intracellular ROS levels	184
6.2.4.4 Determination of cyclobutane pyrimidine dimers (CPD).....	185
6.2.5 Flow cytometry/FACS protocol.....	185
6.2.5.1 Preparation of single cell suspensions from murine skin, wounds, and bone marrow	185
6.2.5.2 Flow cytometry.....	186

6.2.6 Epidermis and dermis separation by heat shock.....	186
6.2.7 Statistical analysis	187
6.3 References.....	187
7. Appendix.....	189
7.1 Abbreviations.....	189
7.2 Curriculum Vitae.....	193
7.3 Publications.....	195
7.4 Acknowledgements.....	197